Some Issues in Data Collection

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Plan for the Lecture

- Talk a little about geometry of diffraction.
- The sources of x-rays and how we handle them.
- Describe screenless rotation.
- What are "partial" reflections and what reflections are missing?
- How do we reduce data?

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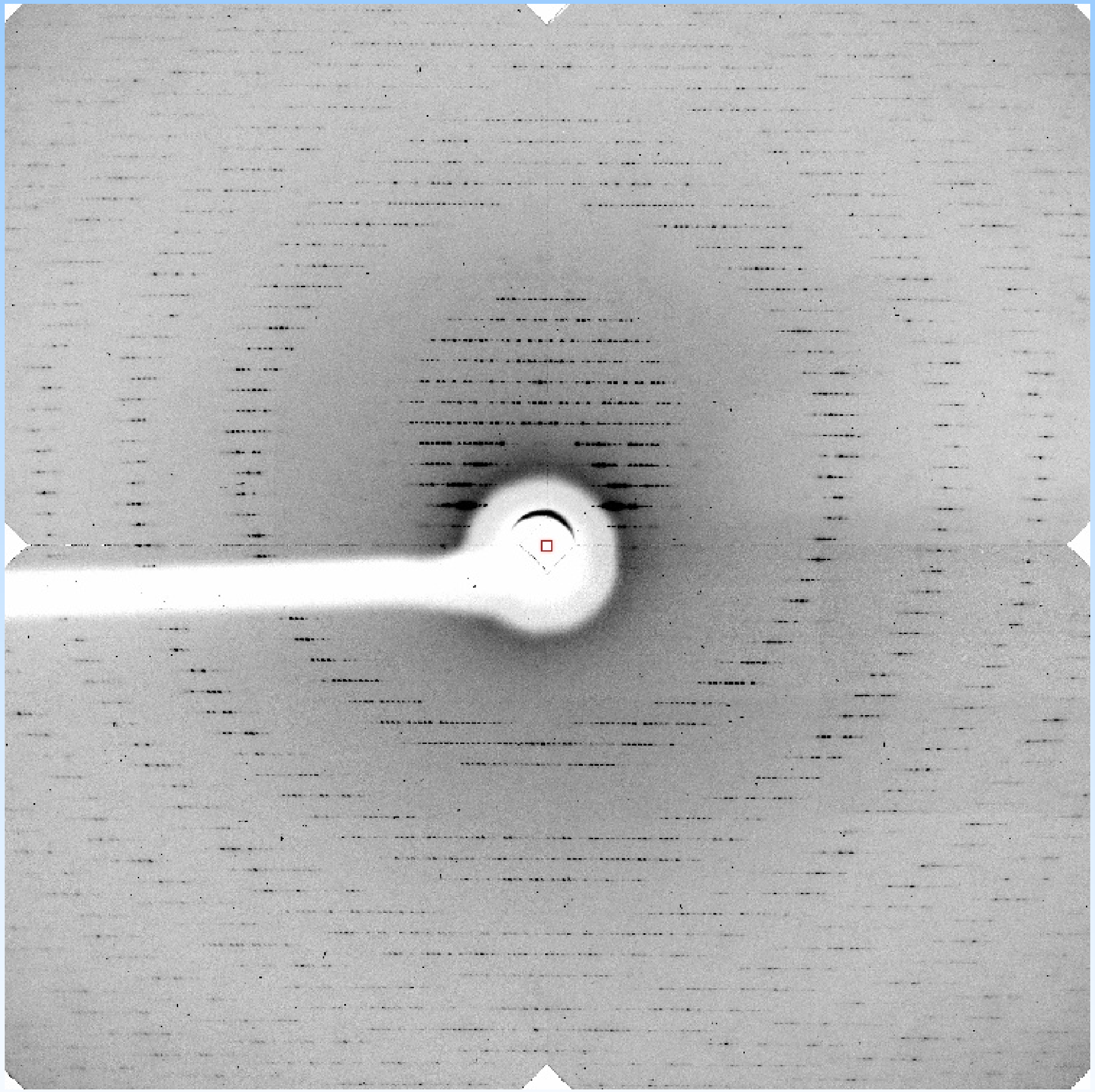
What are the goals of the crystallographic experiment?

- Bring all Bragg planes into diffracting position (**survey all of reciprocal space**).
- ***Integrate*** the diffraction intensity from each set of Bragg planes
  - Over the **volume** of the crystal
  - Over the **crossfire** of the x-ray beam
  - Over the **mosiac** nature of the crystal
- To accomplish this, the reflected beams must be ***resolved***:
  - On the **surface** of the x-ray detector
  - Throughout any **rotation** of the crystal

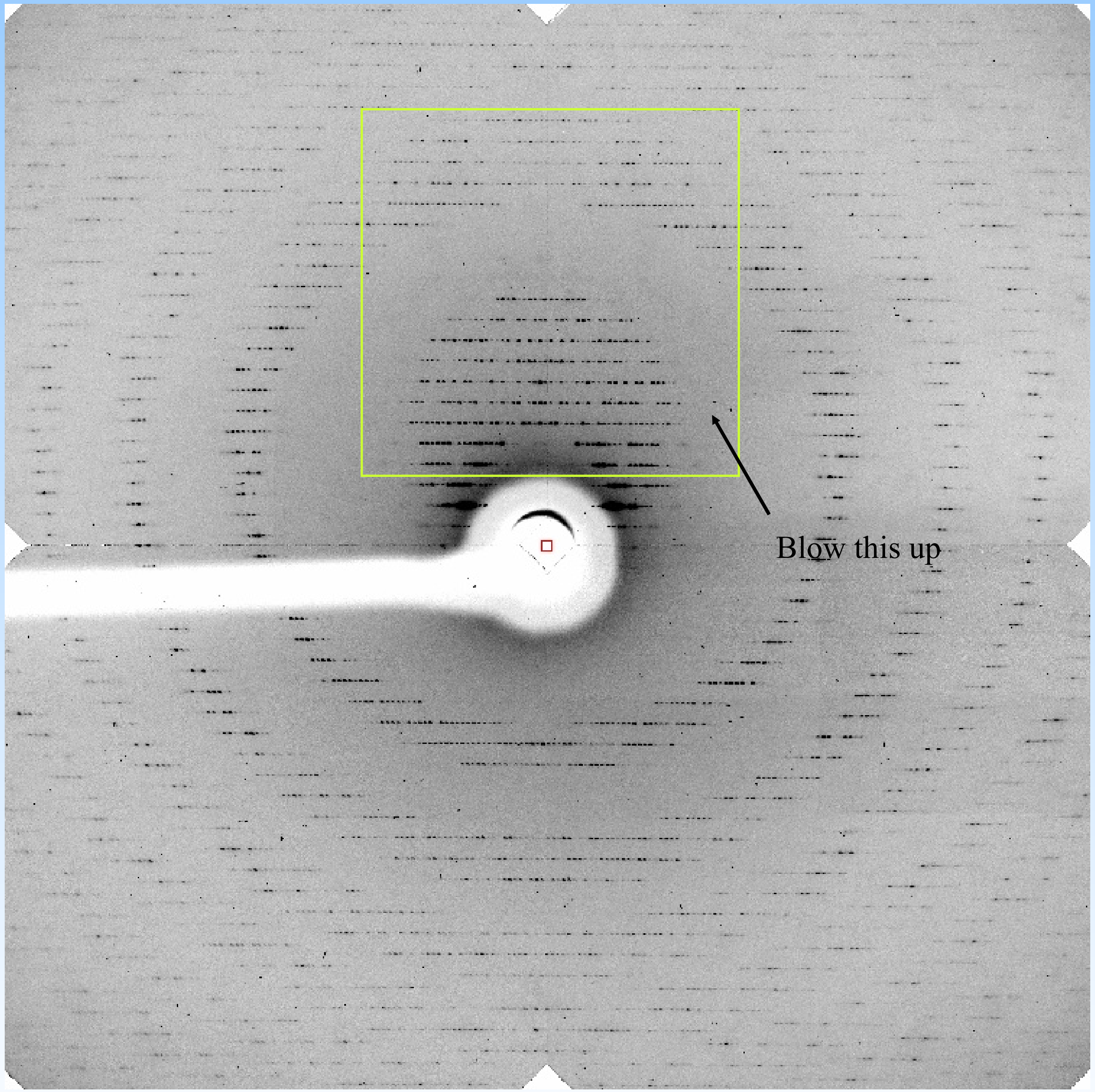
Here's an example of the problem!

The longest unit-cell edge is 570Å in this photo from crystals of the 50S ribosomal subunit.



Ban, et al, Cell 1998

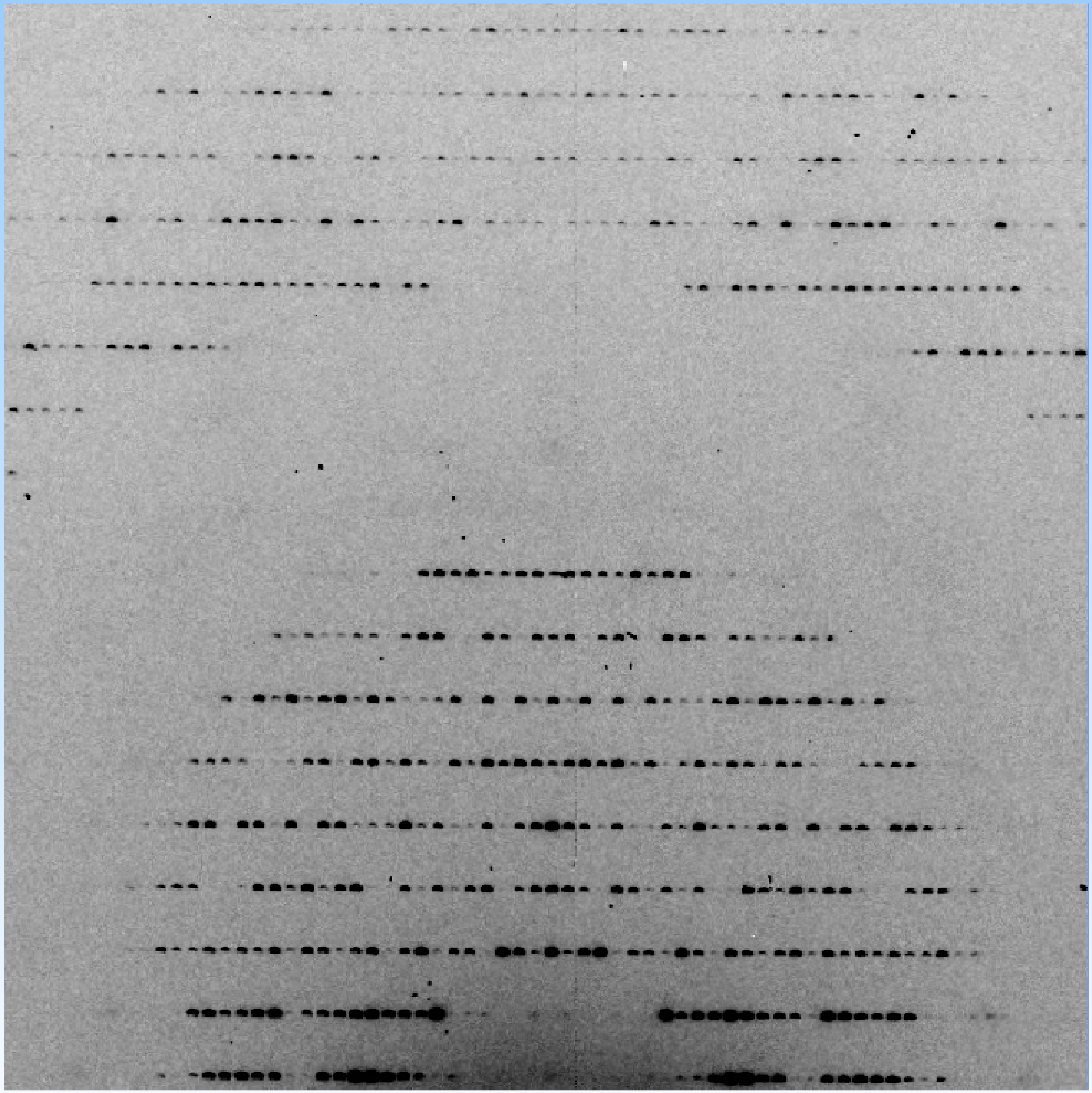
Let's look at it in detail:



Ban, et al, Cell 1998

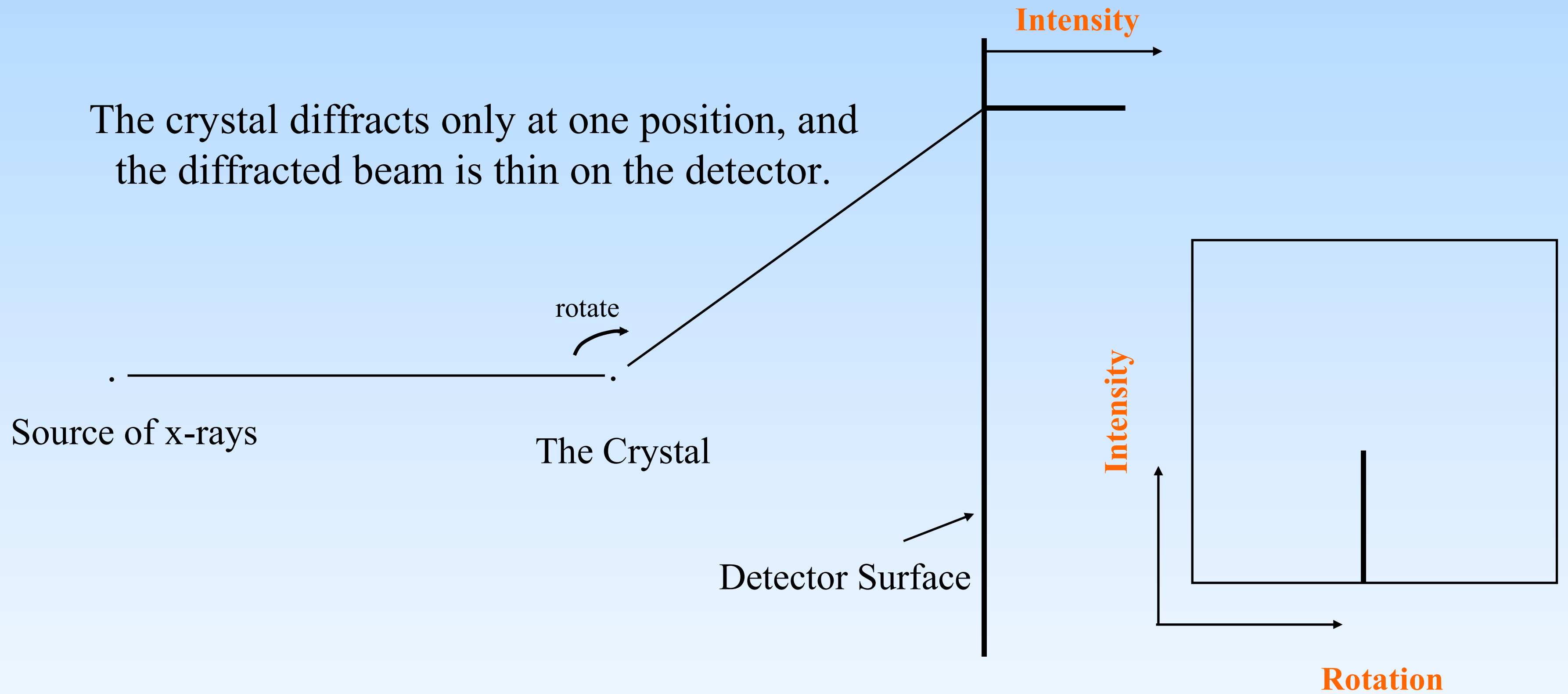
The spots almost touch.

- Their length comes from the horizontal divergence of the focussed beam
- The kidney shape comes from defects in the optics.



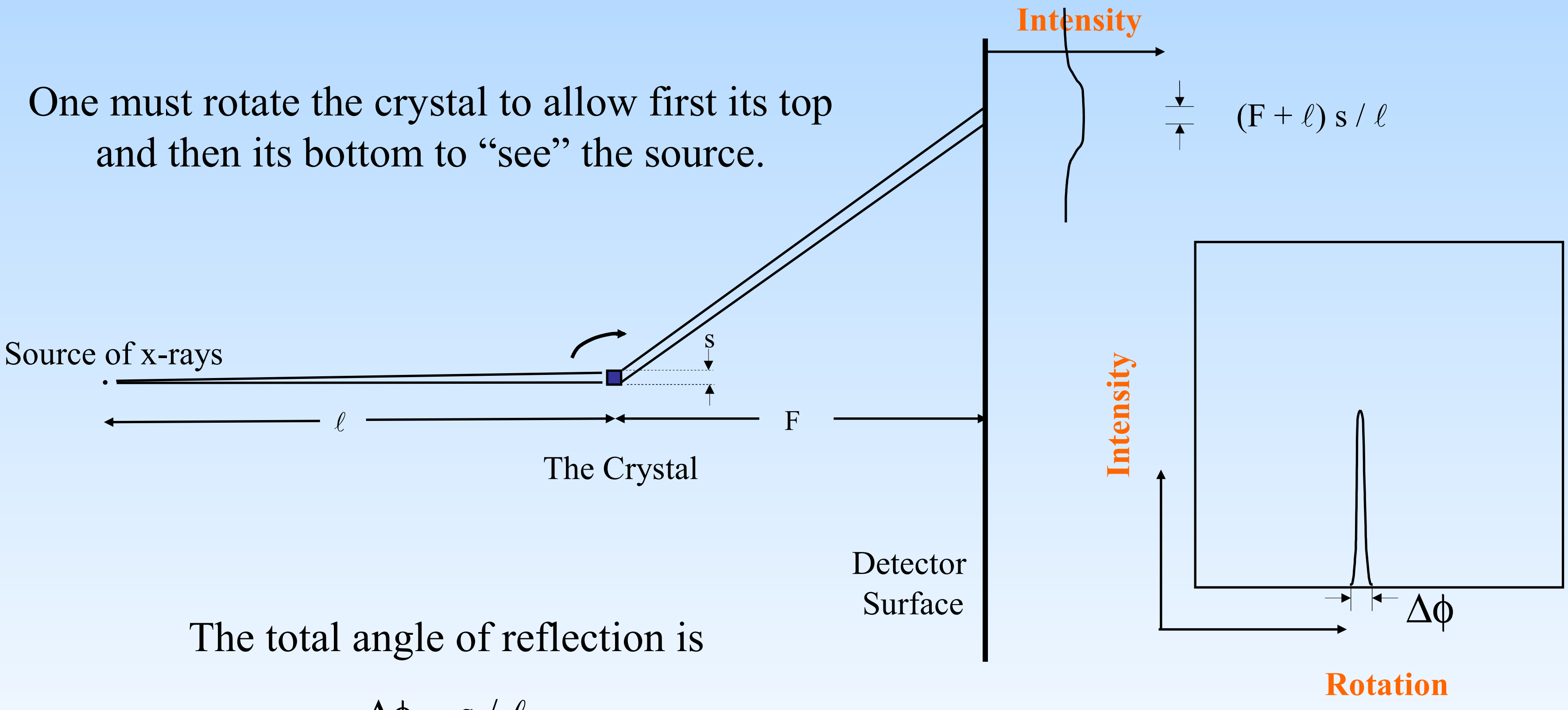
Ban, et al, Cell 1998

Consider the diffraction one would get from a **perfect** infinitesimal (**point**) crystal and a **point** source of x-rays.



Next, consider diffraction from a **finite** perfect crystal and a **point** source of x-rays.

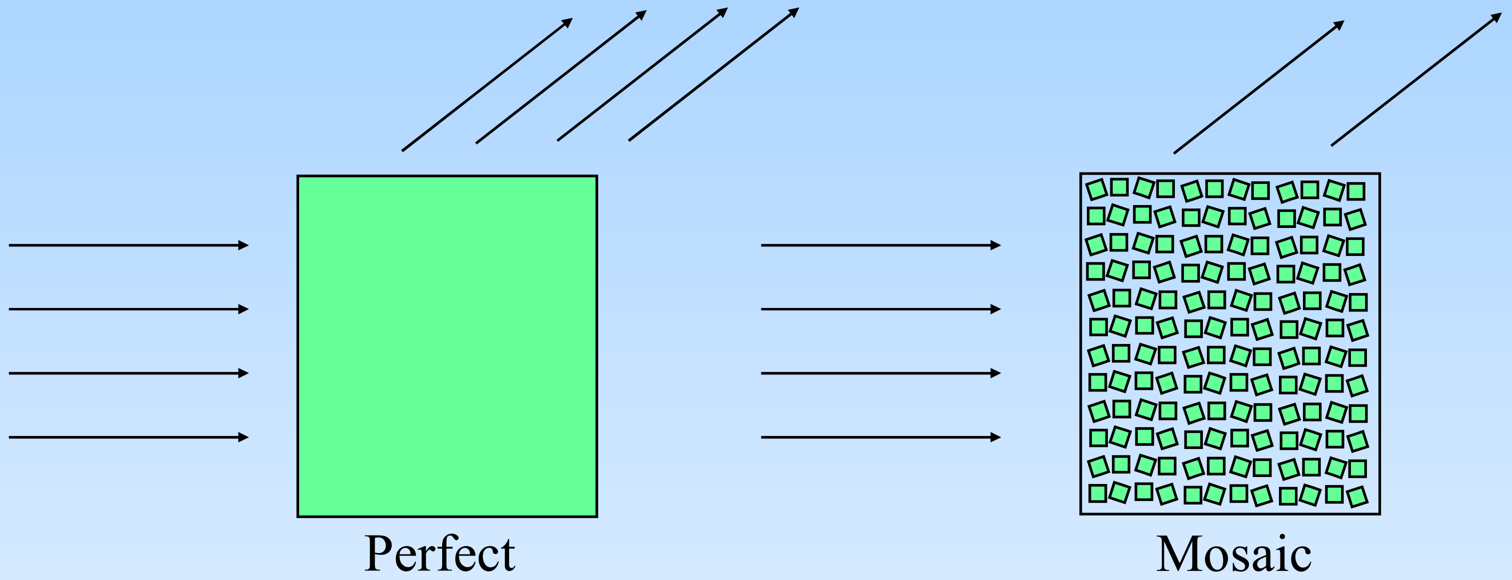
One must rotate the crystal to allow first its top and then its bottom to “see” the source.



The total angle of reflection is

$$\Delta\phi = s / \ell$$

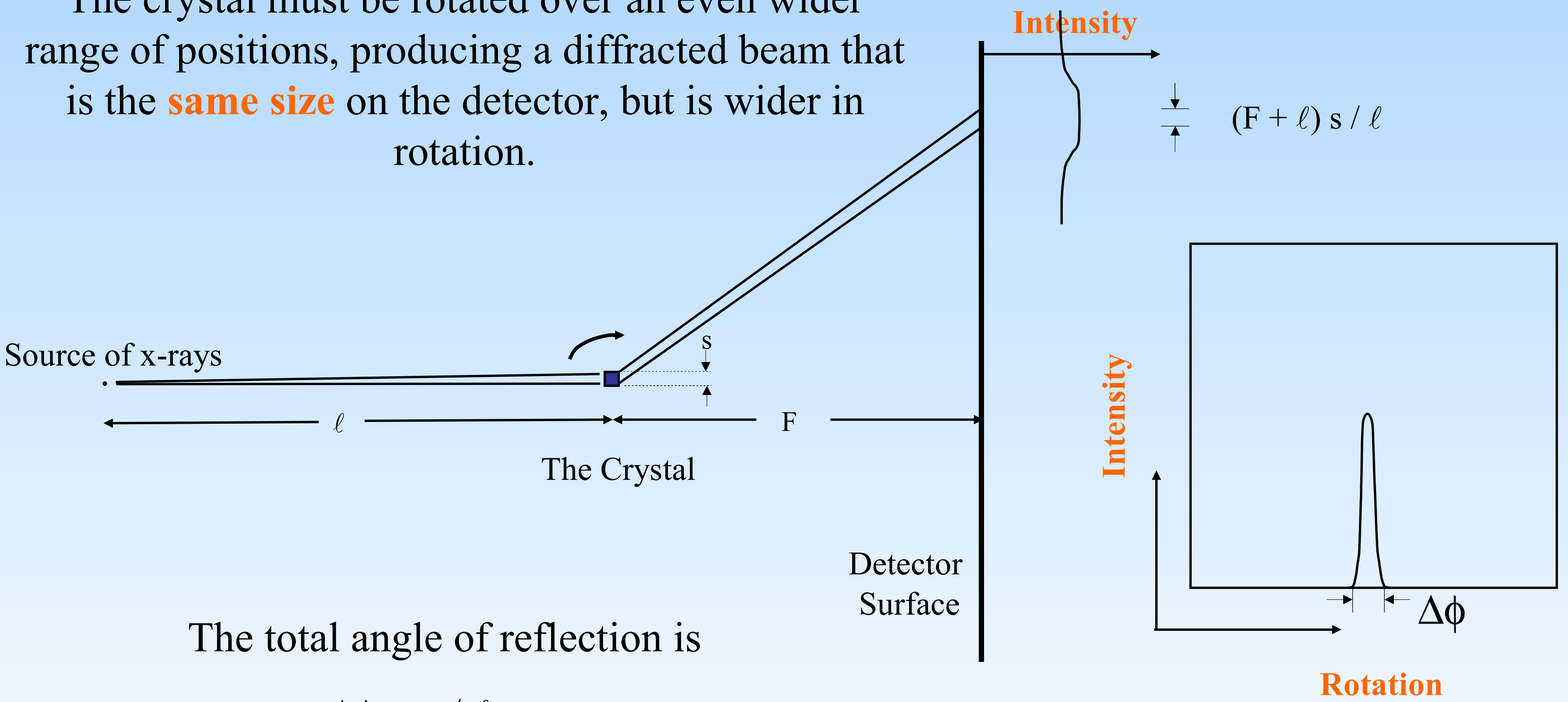
There are perfect crystals, and then there are mosaic crystals



- If the rays coming in are perfectly parallel, the whole block of the **perfect** crystal will diffract, but only those blocks of the **mosaic** crystal that are aligned with the beam will diffract.
- To get all of the mosaic crystal to diffract, one must:
  - Use an x-ray beam with crossfire, or
  - Rotate the crystal in the beam.

Now, consider diffraction from a finite **mosaic** crystal and a **point** source of x-rays.

The crystal must be rotated over an even wider range of positions, producing a diffracted beam that is the **same size** on the detector, but is wider in rotation.

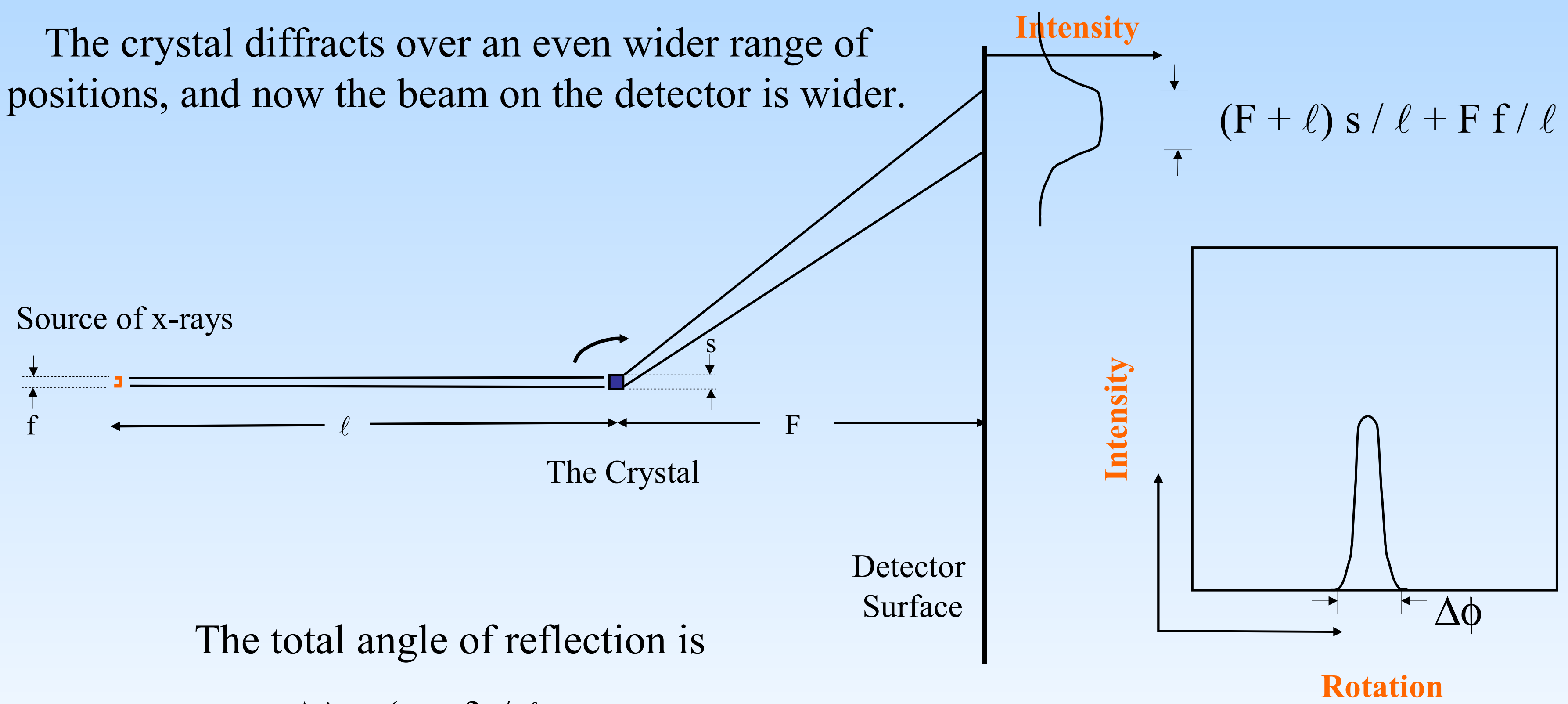


The total angle of reflection is

$$\Delta\phi = s / \ell + \mu$$

Finally, consider a finite **mosaic** crystal and a **finite** source of x-rays.

The crystal diffracts over an even wider range of positions, and now the beam on the detector is wider.



The total angle of reflection is

$$\Delta\phi = (s + f) / \ell + \mu$$

Let's think about spot size and spot separation.

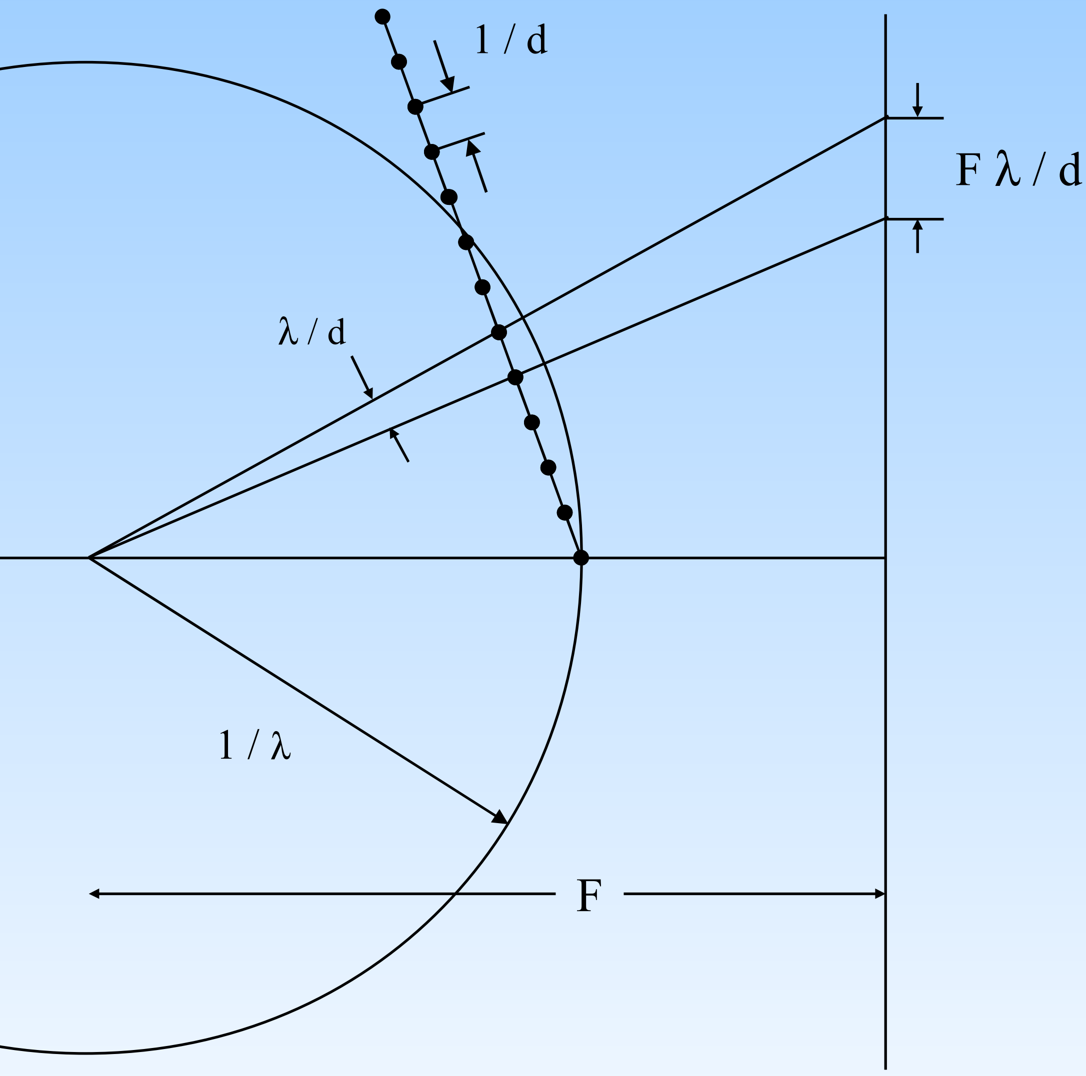
On the previous drawing we had that the spot size was:

$$(F + \ell)\, s / \ell + F\, f / \ell = s + F\,(s + f) / \ell$$

Notice that this $(s + f) / \ell$ term represents all of the crossfire of the beam, whatever the source

We'll call it γ.

So the spot size becomes $\mathbf{s + \gamma\, F}$



Therefore, to resolve spots, we must have that the spacing is greater than the size:

$$F \lambda / d > s + \gamma F$$

$$F [\lambda / d - \gamma] > s$$

We can accomplish this by increasing F until it works:

$$F > s / [\lambda / d - \gamma]$$

One can see that this will work as long as the beam crossfire is small enough!!

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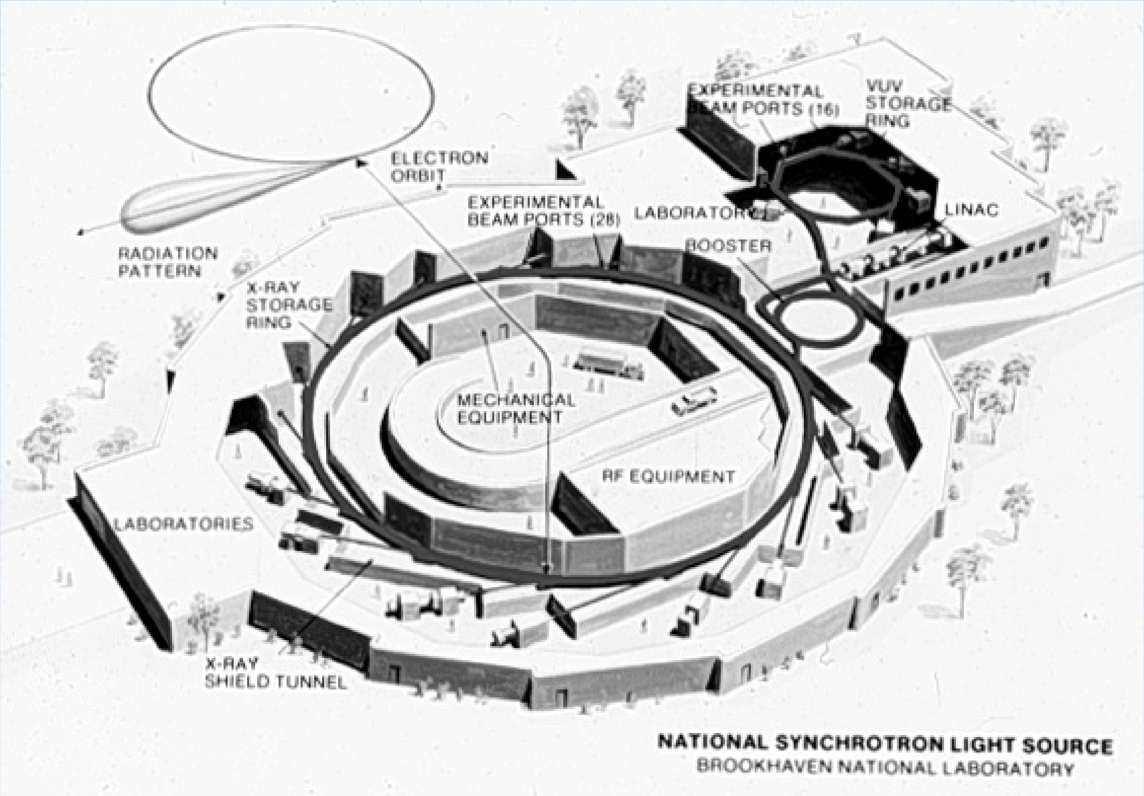
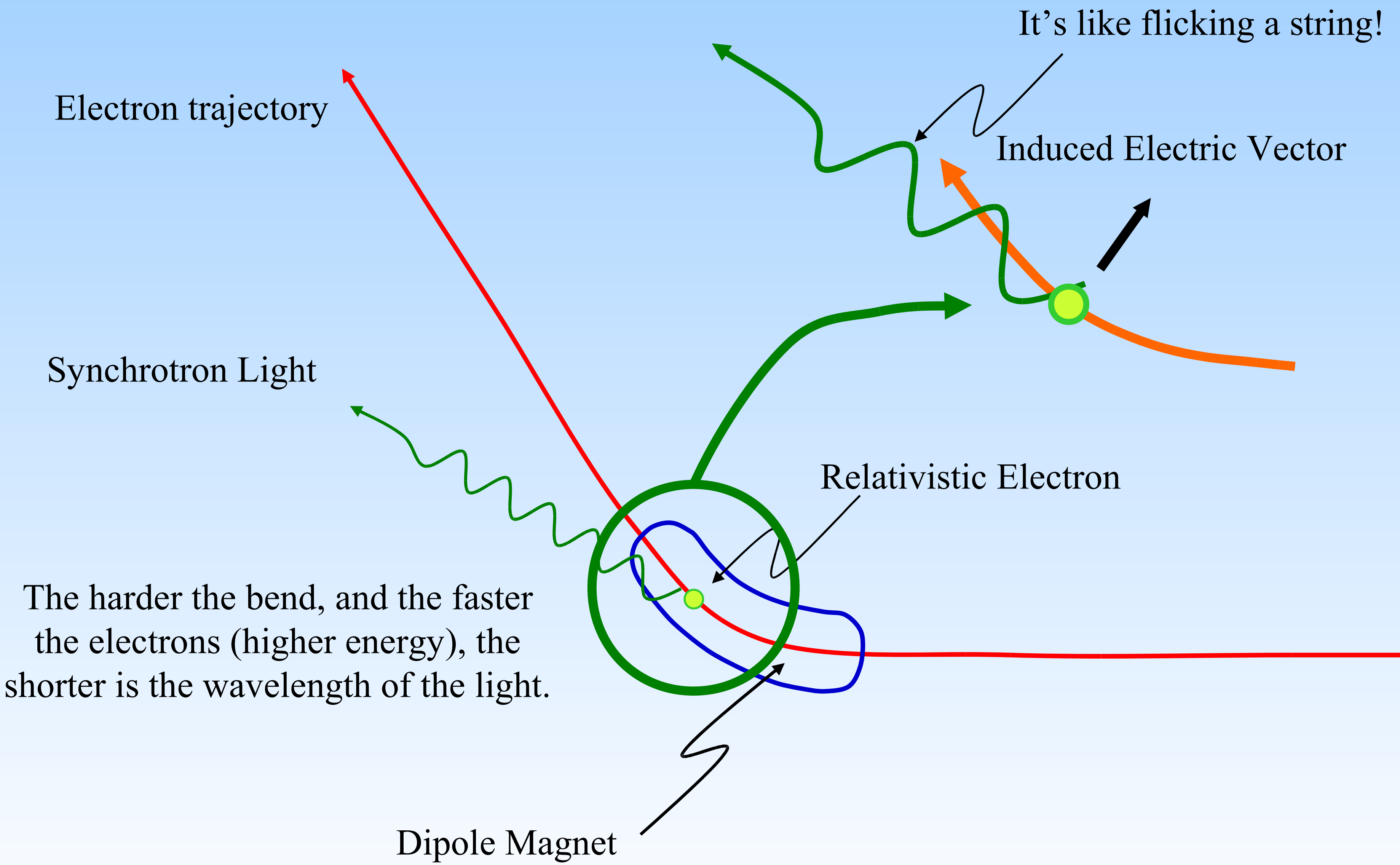
The National Synchrotron Light Source

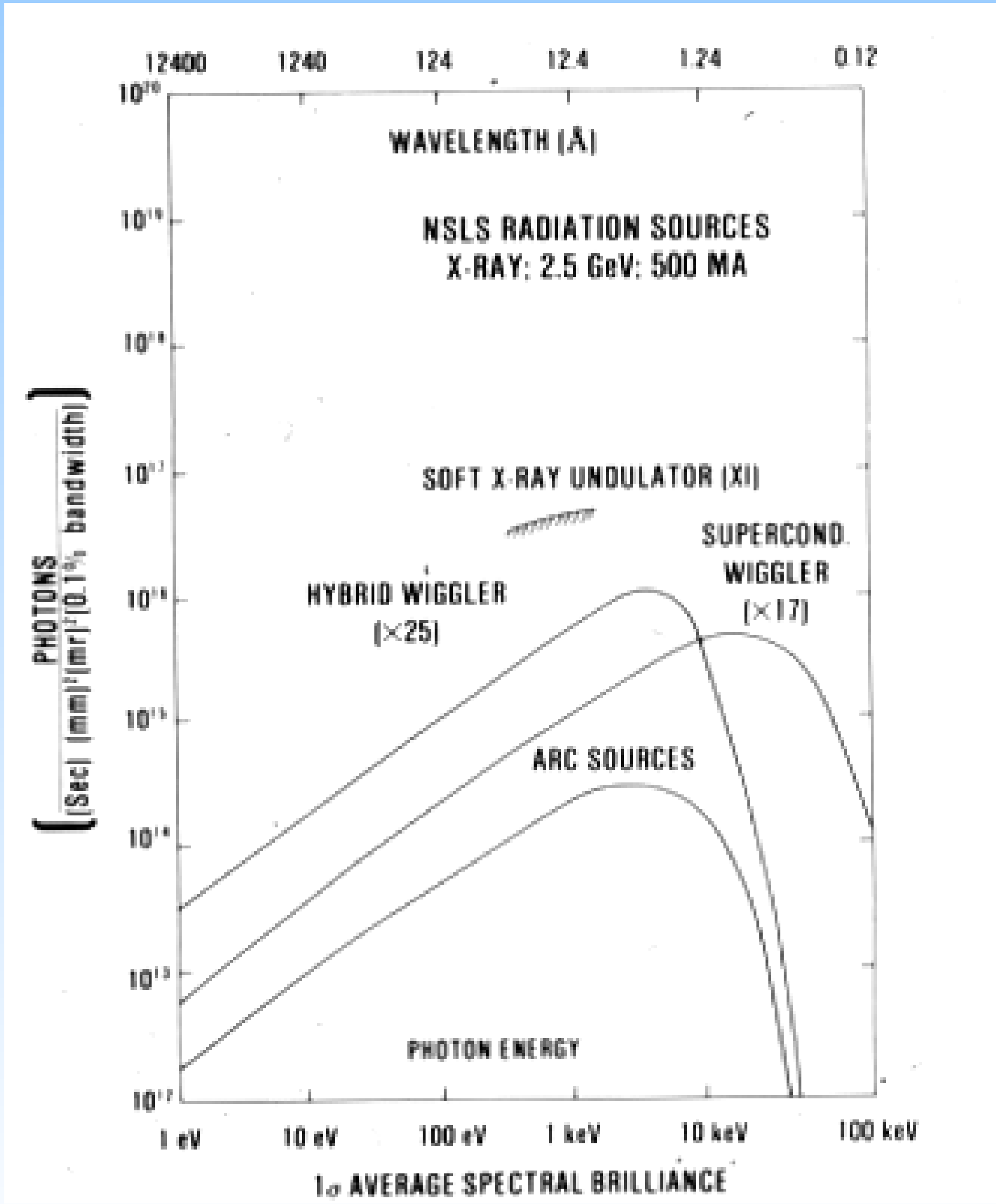


The NSLS can produce usable radiation from the dipole, or bending" magnets. There are two storage rings producing radiation. The lower-energy ring (750 MeV) produces light from the IR through to soft X-rays. The higher energy ring (2.6 GeV) produces hard X-rays for diffraction studies. Both rings have multi-magnet insertion devices.

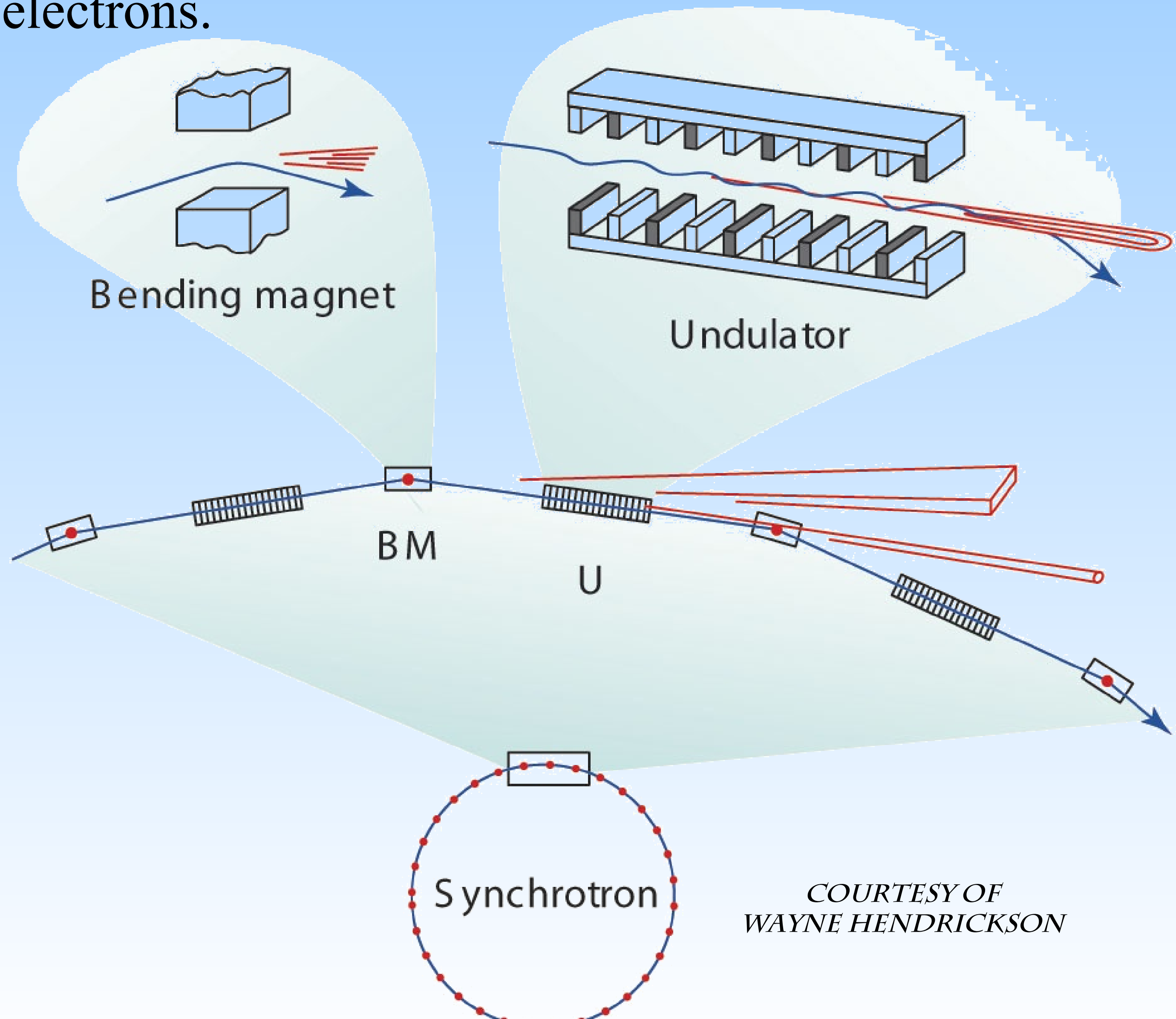
The Source of Synchrotron Radiation



There's a wide spectrum of x-rays available to us for diffraction studies. Here you see the arc (dipole or bending-magnet) and wiggler sources.

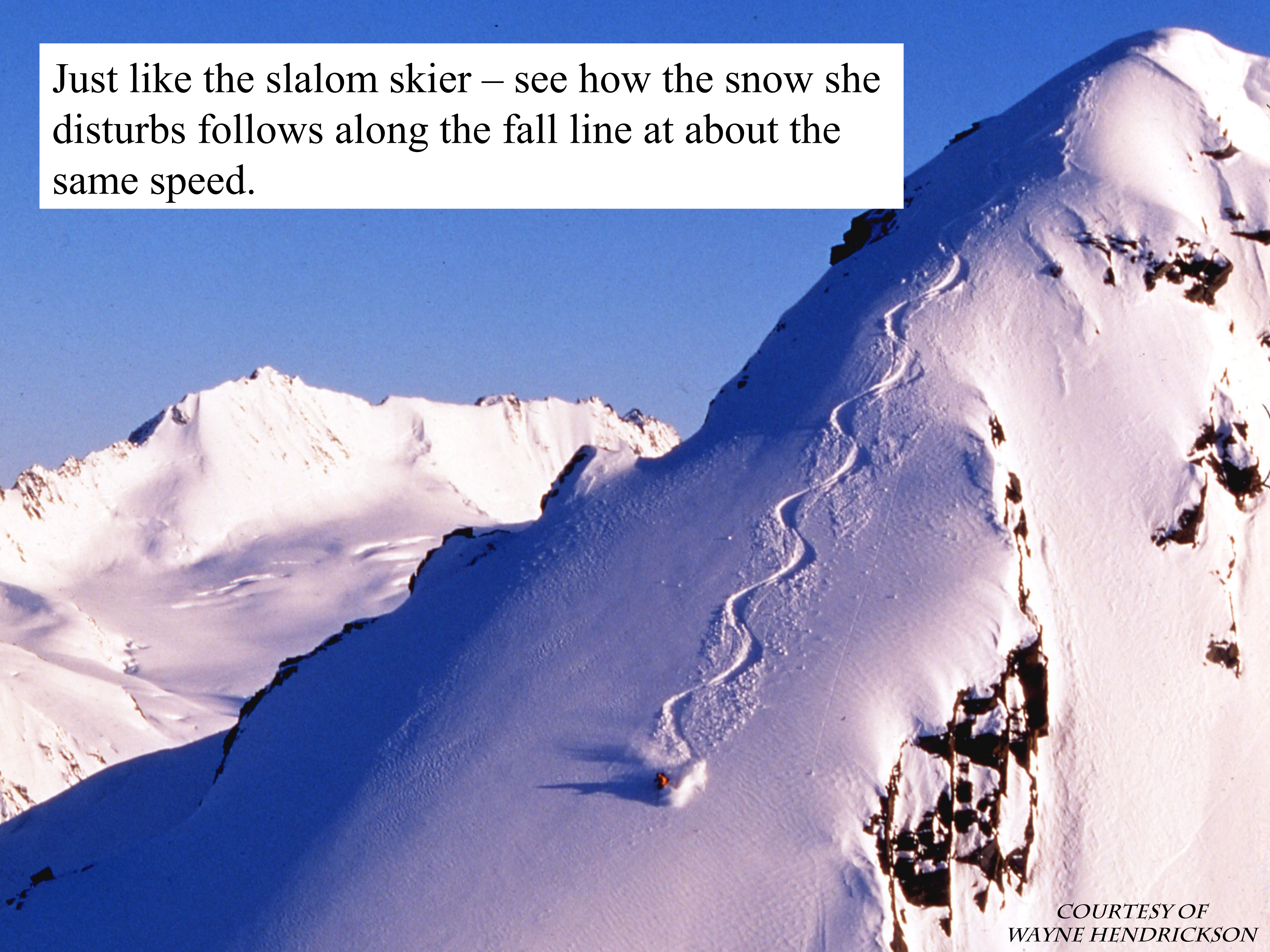


This is for dipoles (bending magnets). For undulators, the photons emitted at the little dipoles run along beside the (relativistic) electrons.



COURTESY OF WAYNE HENDRICKSON

Just like the slalom skier – see how the snow she disturbs follows along the fall line at about the same speed.



COURTESY OF
WAYNE HENDRICKSON

The photons interfere with the electrons, and at each undulation the electrons produce new photons that are a harmonic of the frequency of the original, thus the peaky spectrum you see here.

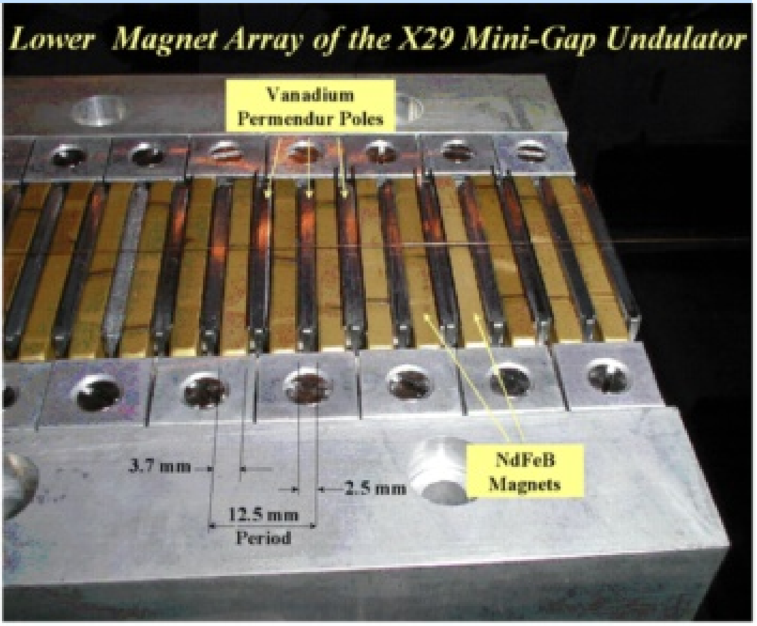
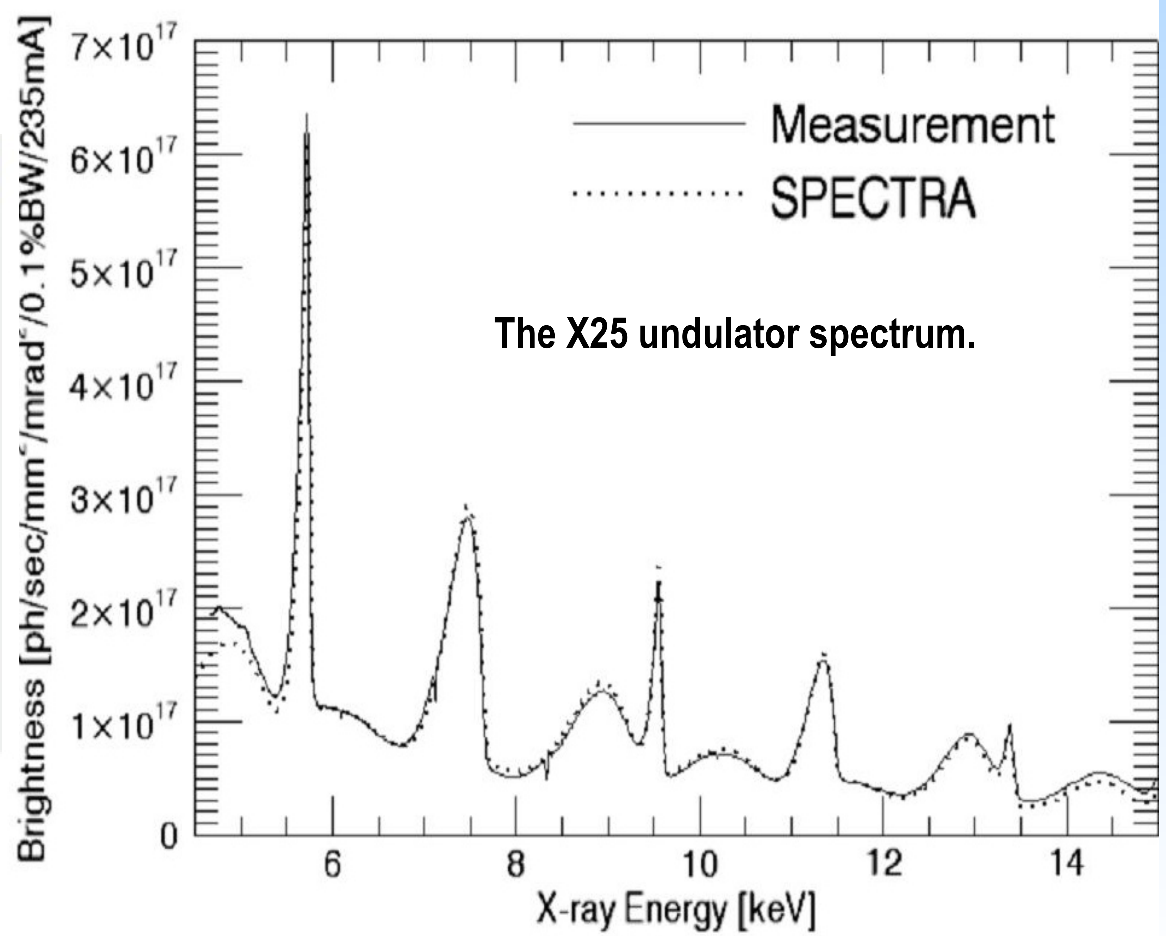
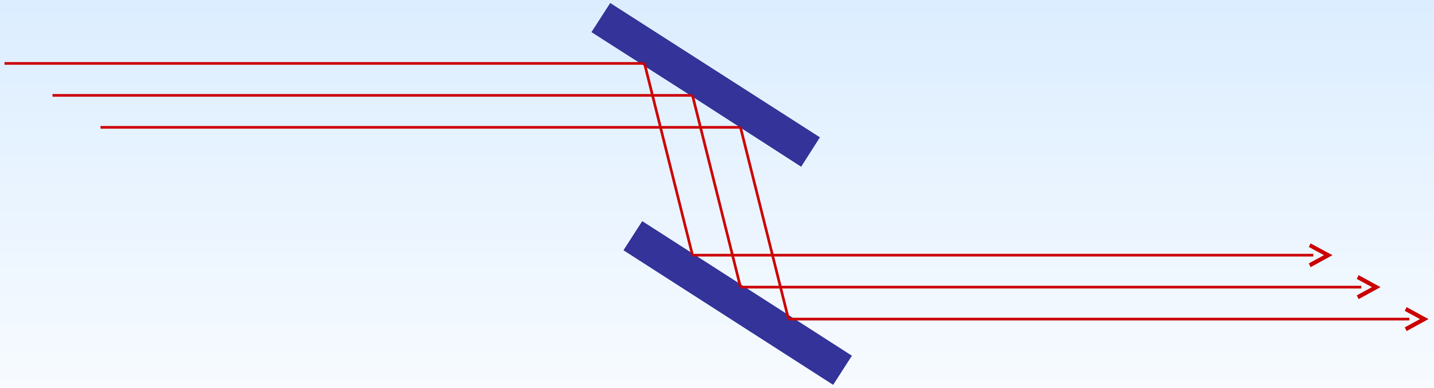


Figure 1



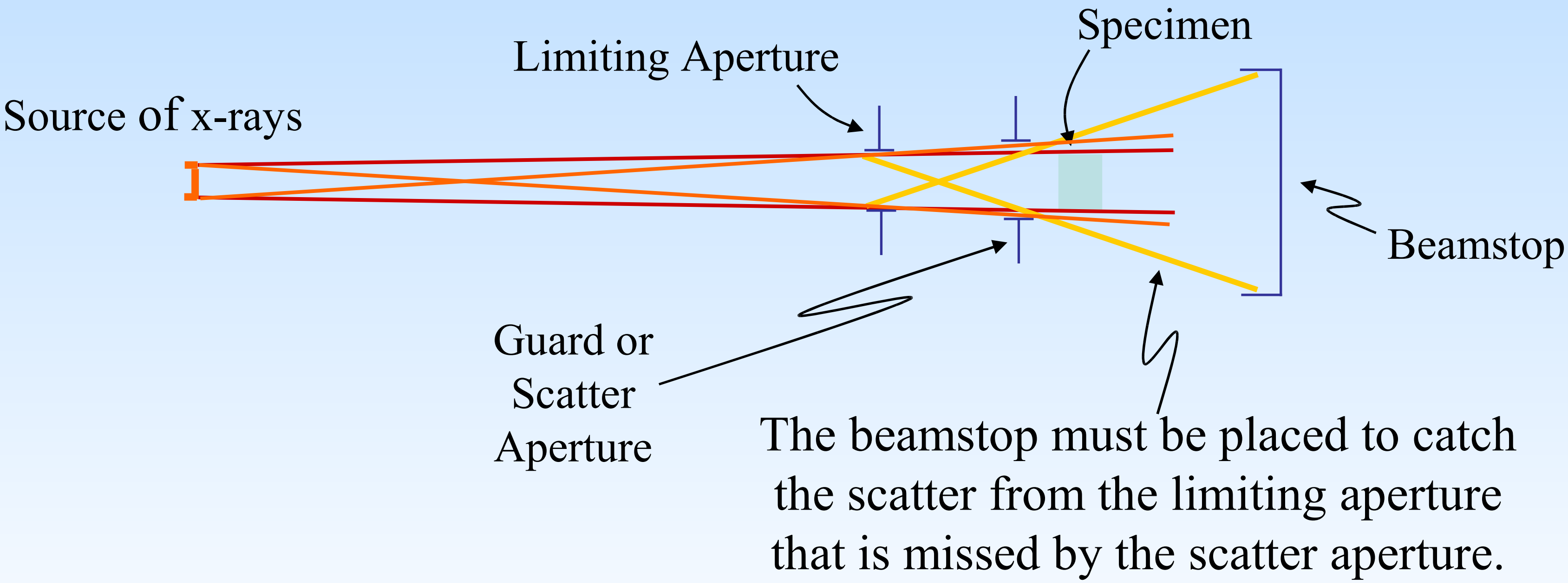
For a synchrotron source:

- Rays are nearly parallel
- Use a perfect crystal
- Si cut to use the (1,1,1) planes is a good choice
- Often we use two crystals:
  - One to monochromatize
  - One to deflect the beam back up into the horizontal plane
- Braggs' law determines the wavelength



Beam Collimation (Use of Slits)

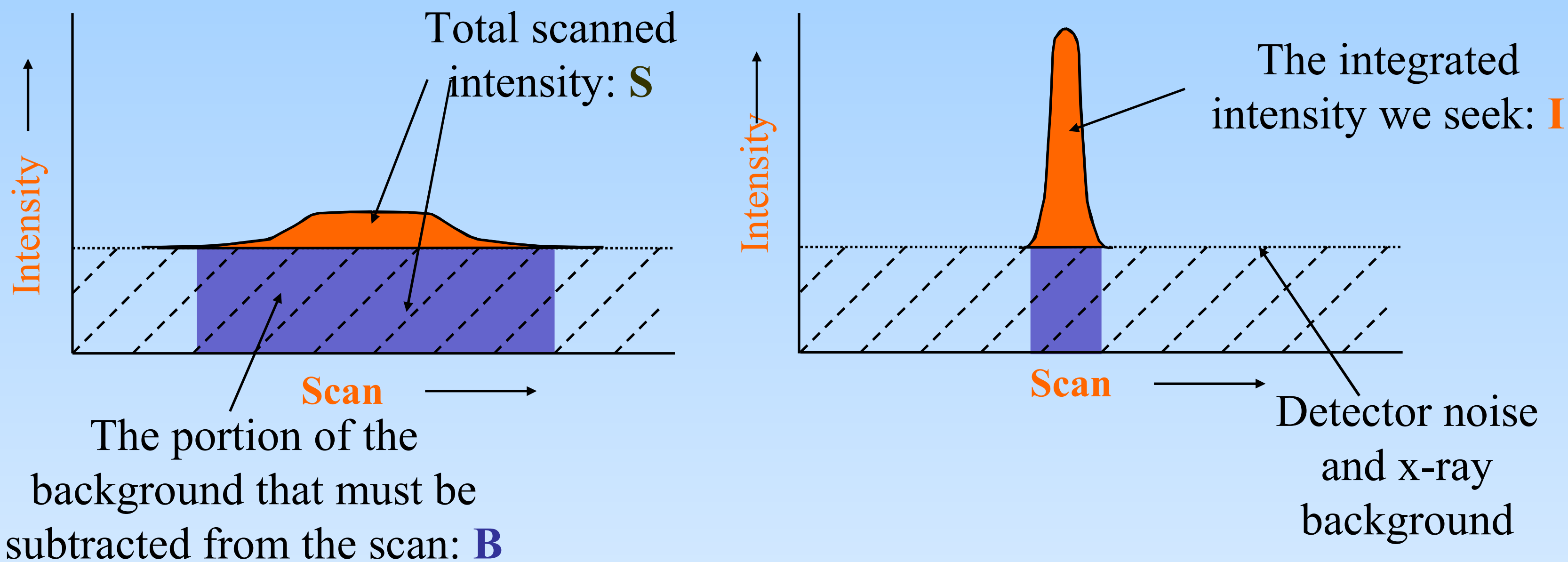
- In a classic sense, collimation means “to make the rays parallel.”
- We generally use the term to mean limiting the beam and avoiding extra scattered rays.



Accuracy - I: Statistical Precision

- With photon counting, var (I) = σ^2 (I) = I
  - For example, relative error is $\sigma(I)/I = 1/I^{1/2}$
  - Therefore, for $I = 10^4$, $\sigma(I)/I = 0.01$ (1% error)
  - There must be **sufficient photons** to provide the statistical precision one wants.
- The **signal** must be strong enough to **rise above** the noise in the counting system and other sources of "**background**" noise.

Consider two different situations:



- We have: **I** = **S** - **B**
- Standard error propagation gives $\sigma^2_I = \sigma^2_S + \sigma^2_B$
- Since I, B, and S all are counts, $\sigma^2_S = S$, $\sigma^2_B = B$
- This gives: $I / \sigma_I = (S - B) / (S + B)^{1/2}$
- It pays to make the reflection **sharp.**
- One wants a bright source!

Ways to Decrease the Background

- Make the specimen mount as small as possible (***the loop and droplet scatter x-rays***)
- Use a beam no larger than the specimen (***ditto above, plus the extra air in the beam***)
- Place the beamstop as close to the specimen as practicable (***shorten the air path***)
- Move the detector as far from the crystal as possible, consistent with the resolution you desire (***the diffracted beams are collimated, the background goes down as $1/r^2$ from the specimen region***)

Accuracy - II: Minimize Systematic Errors

- Specimen damage:
  - One will see variation in data
  - Resolution limit decreases
  - Usually one can freeze the crystal
  - Synchrotron Rad’n helps to get data fast
- Absorption errors:
  - Watch for high salt solutions
  - Make freezing loops as small as possible
  - Because absorption varies as λ^3 while scattering varies as λ^2, it pays to use a shorter wavelength.

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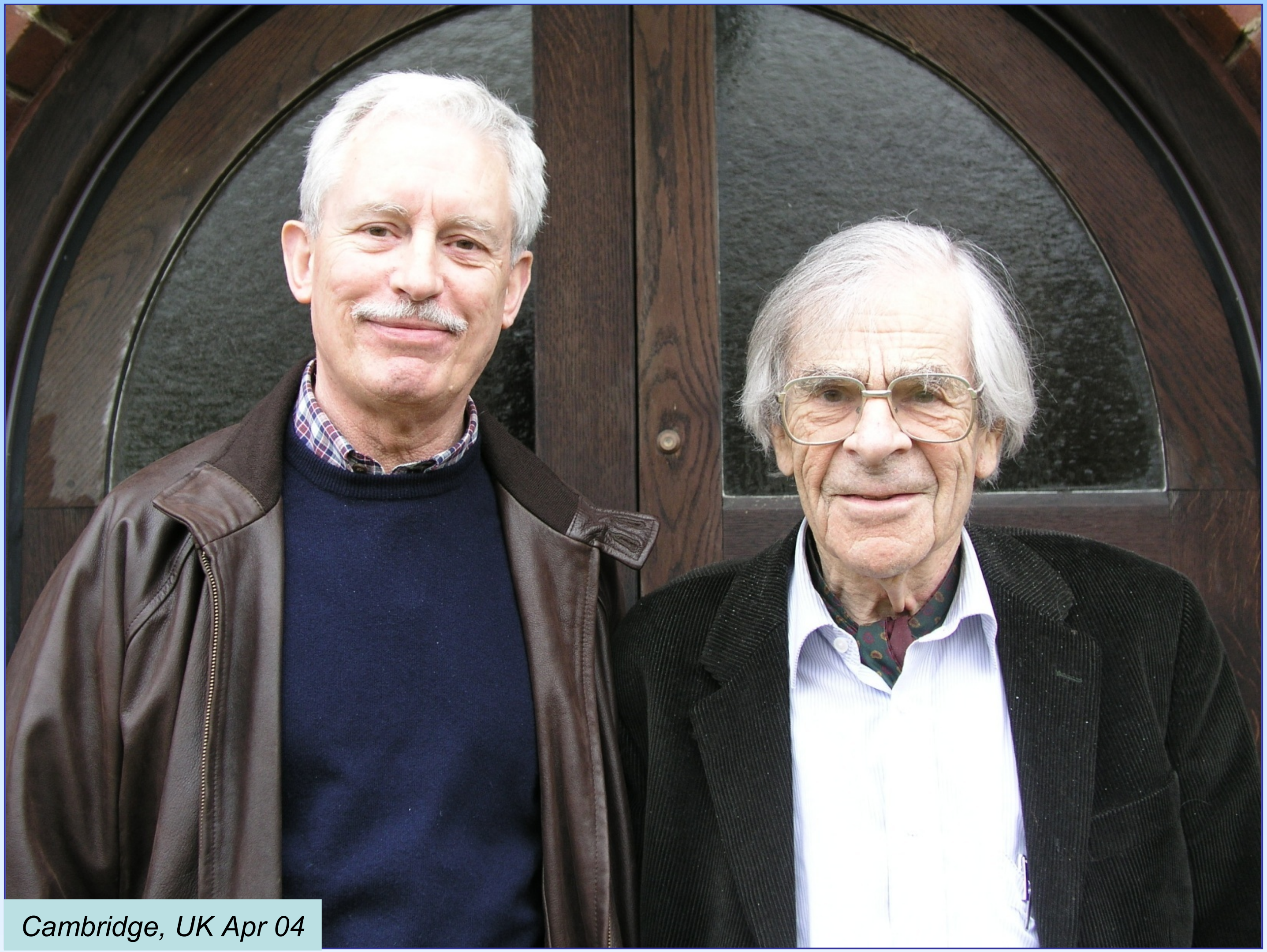
These next comments are taken largely from chapters by Uli Arndt and Alan Wonacott from their classic book:

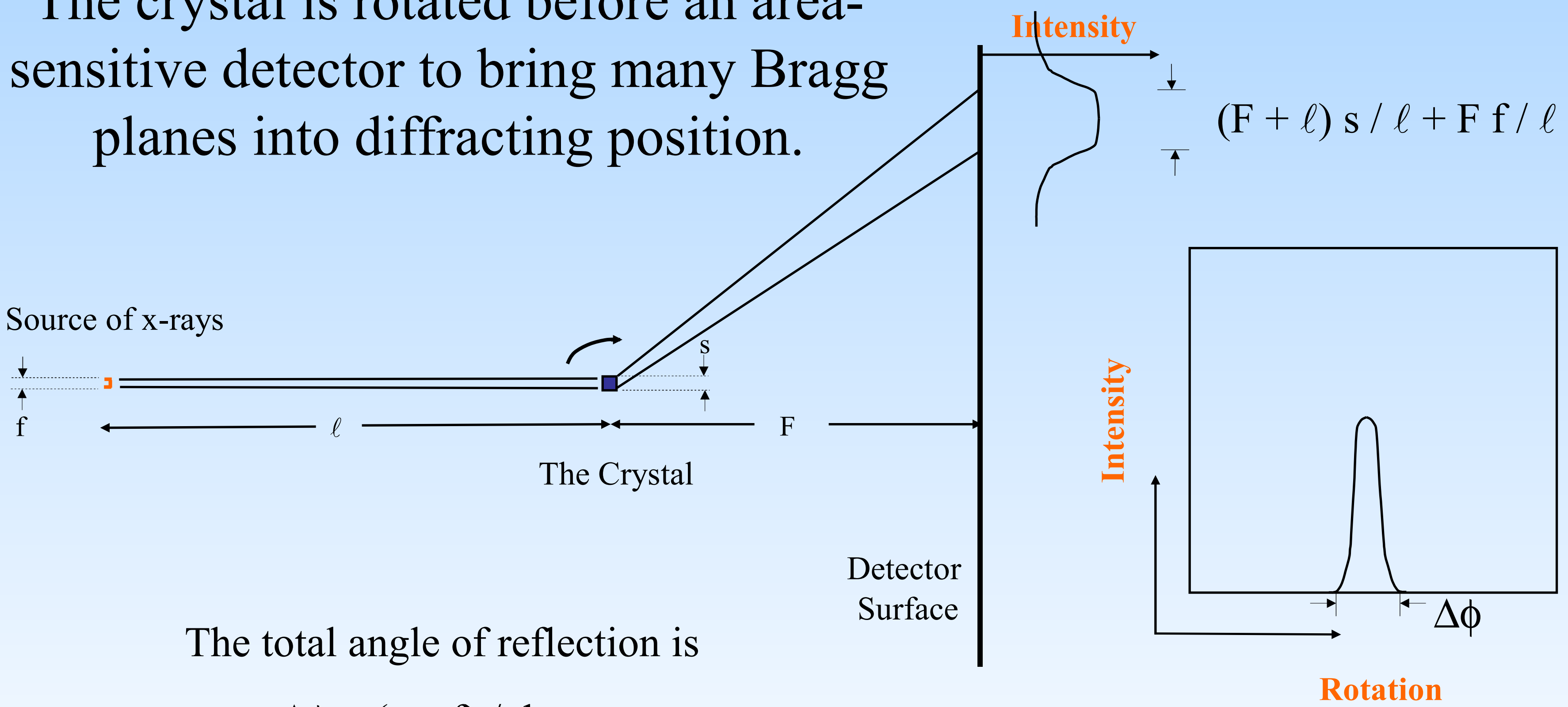
The Rotation Method in Crystallography
Editors: U.W. Arndt and A.J. Wonacott
North-Holland Publishing Co.
Amsterdam, 1977



I am indebted to Alan as a valued friend, teacher, and colleague, and to Uli for all of these things, plus his role as a mentor to me.

You already know how the experiment works

The crystal is rotated before an area-sensitive detector to bring many Bragg planes into diffracting position.

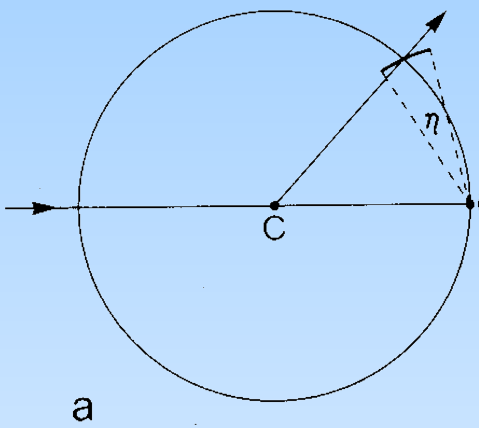


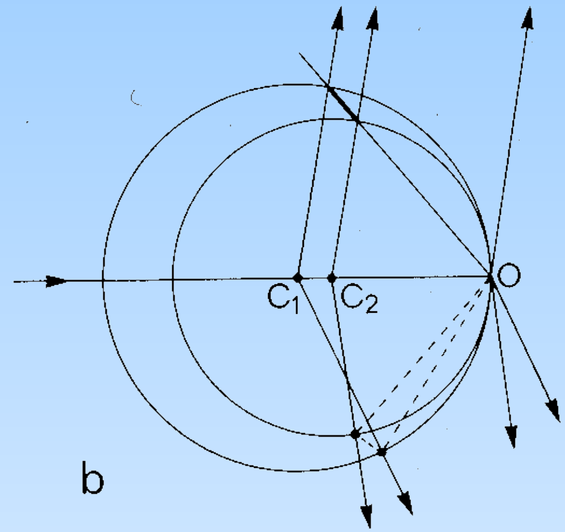
The total angle of reflection is

$$\Delta\phi = (s + f) / d + \mu$$

Various properties of the system will affect diffraction

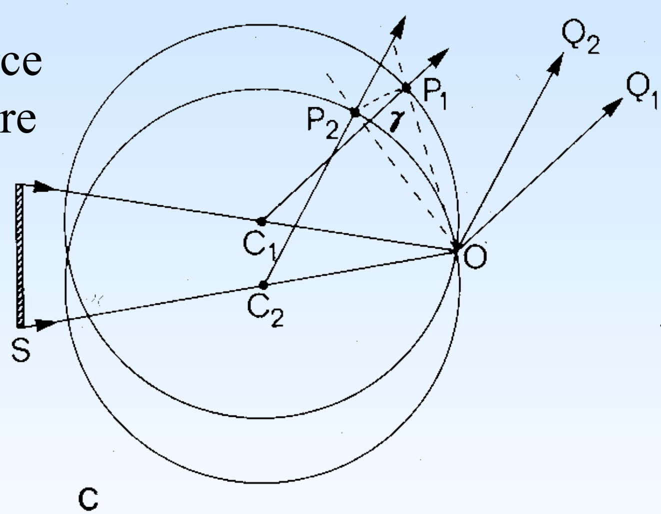
a. mosaic spread

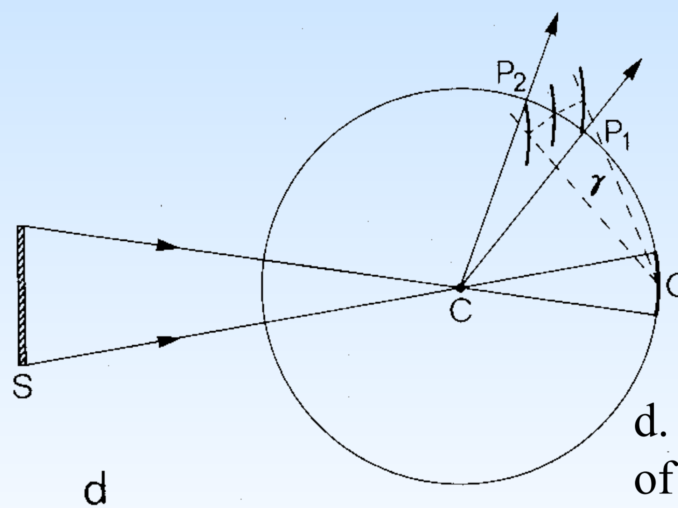




b. Wavelength spread

c. source crossfire

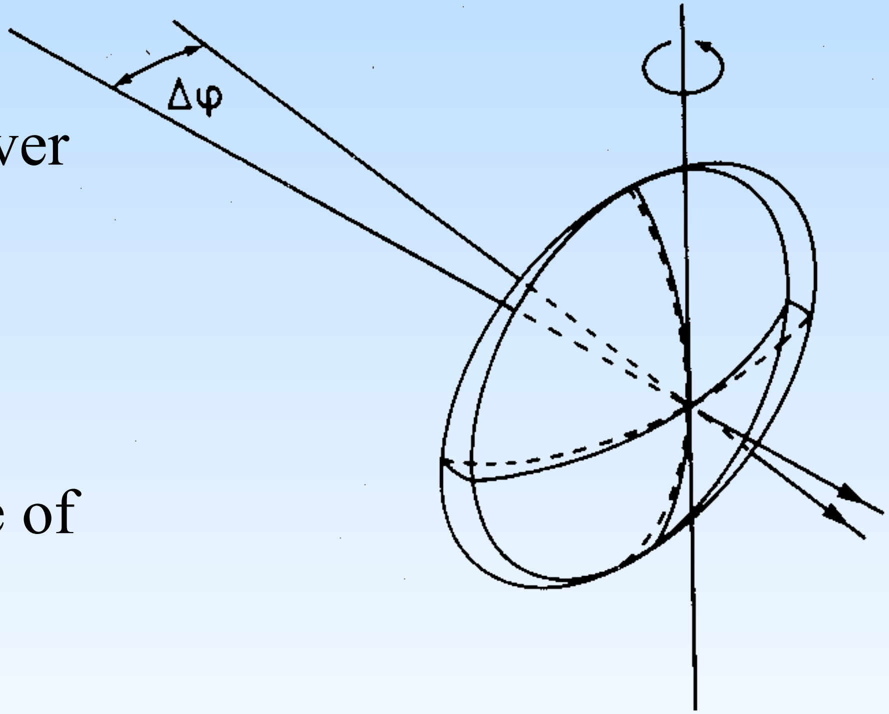




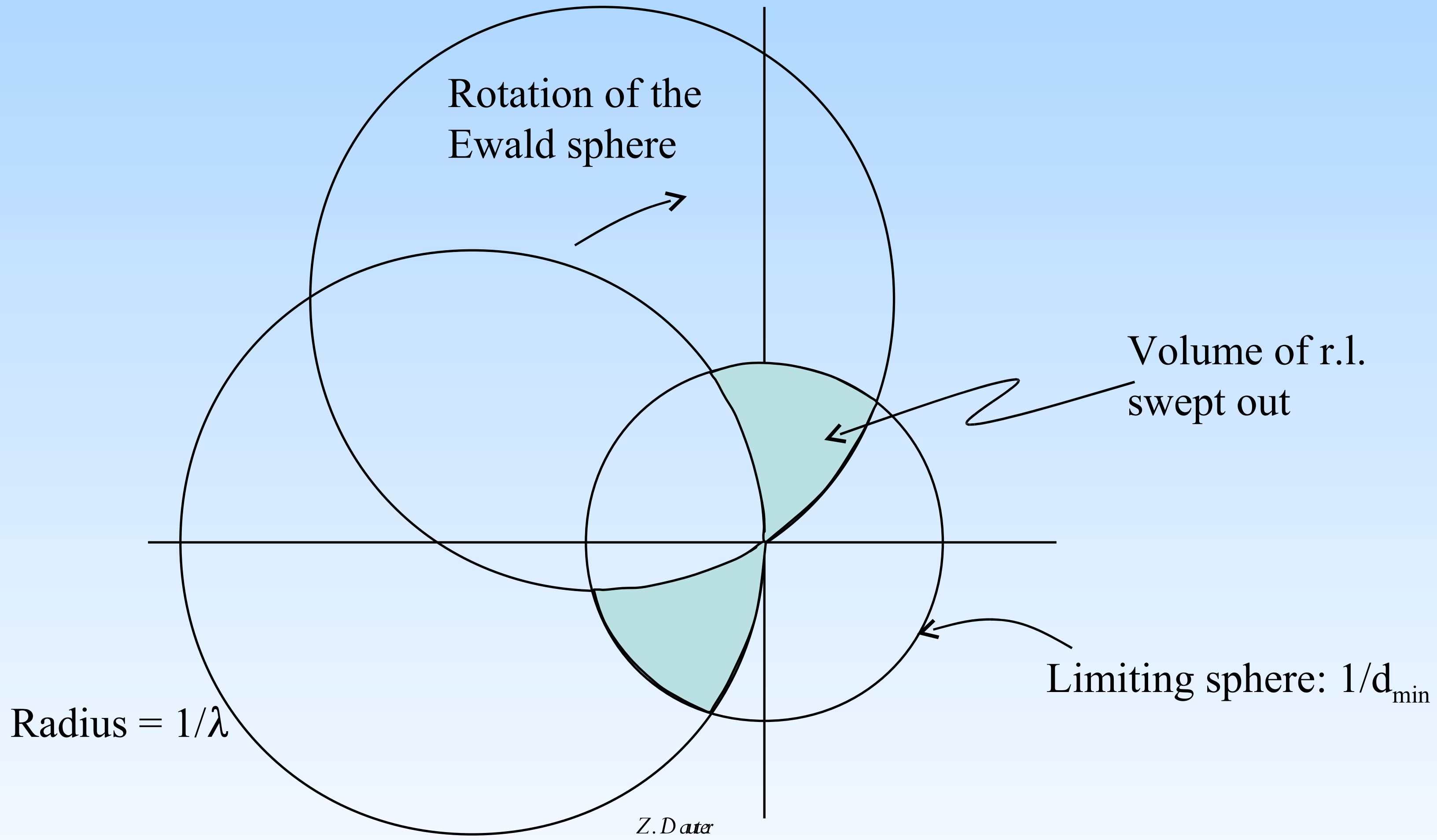
d. another view of source crossfire

Rotation sweeps out a strangely-shaped volume, however...

- Many r.l. points will be recorded during a single short rotation.
- Contiguous rotations will cover much of the reciprocal lattice.
- The "camera" is simple: an axis, a film, and a shutter.
- It's easy to substitute a range of detectors.



One can quickly scan most of the volume of the limiting sphere



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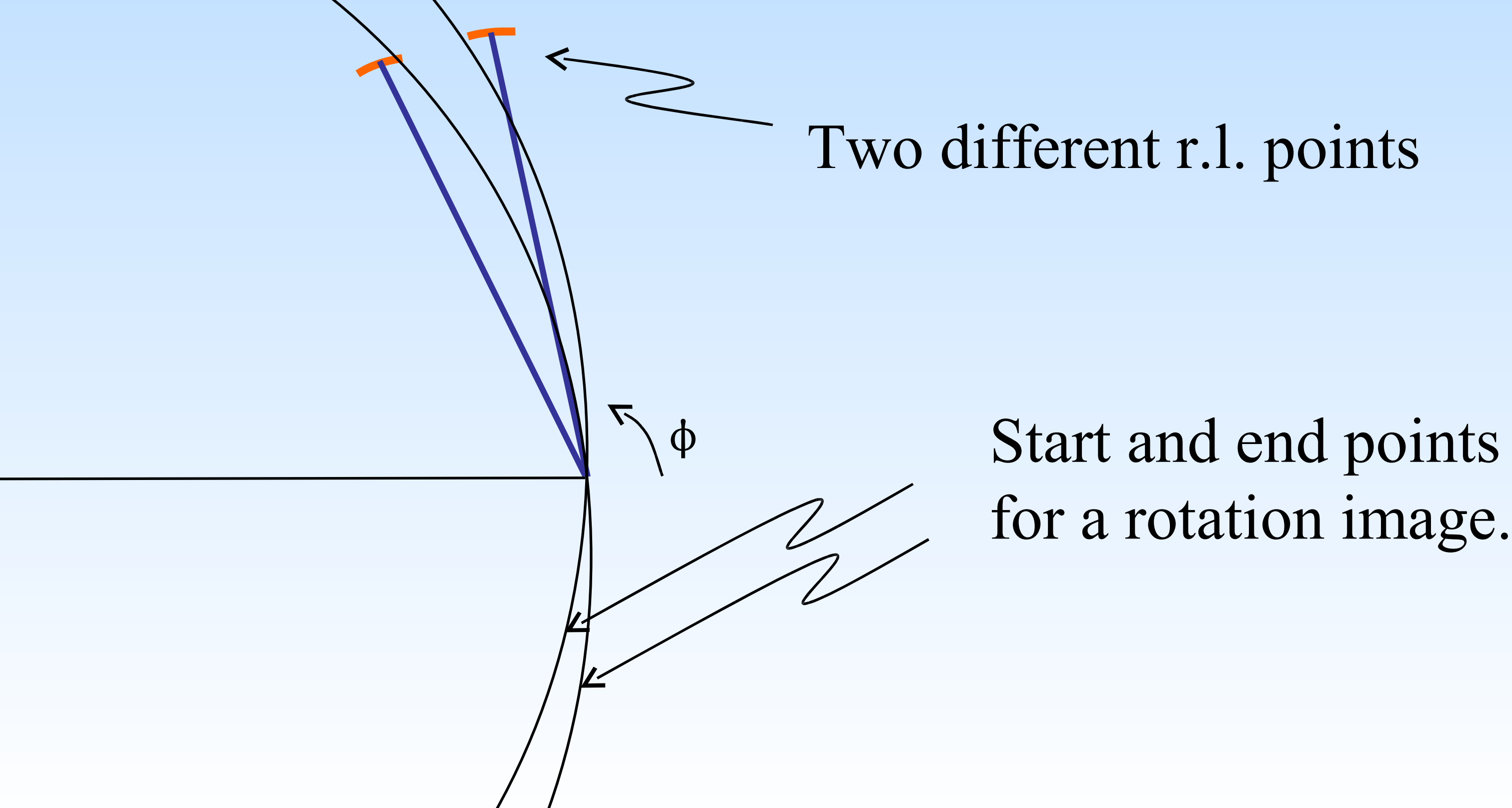
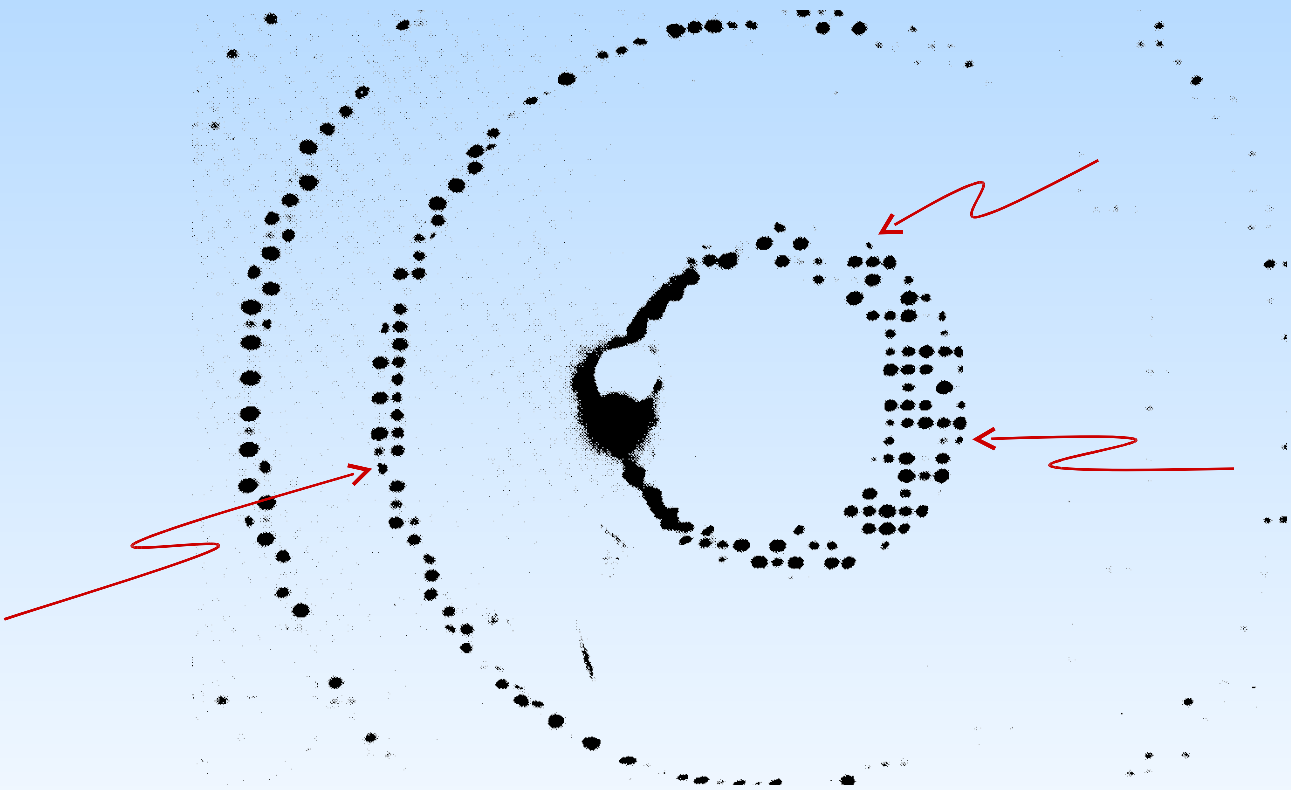
There's a problem, and a solution:

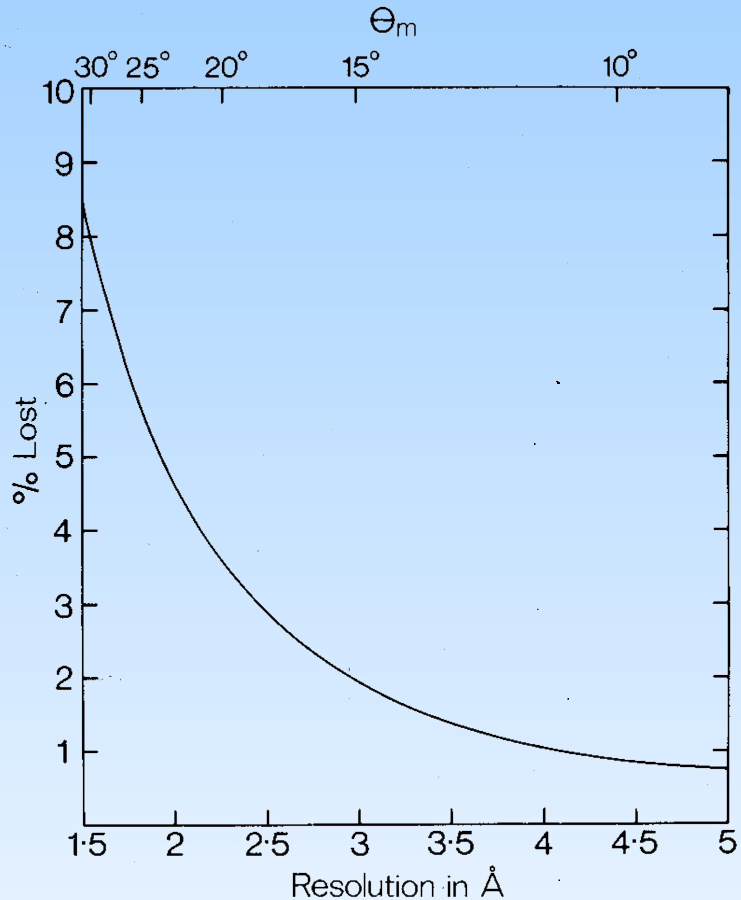
- Reflections can be partially recorded on each of two successive rotations.
- However, the intensities measured can be summed



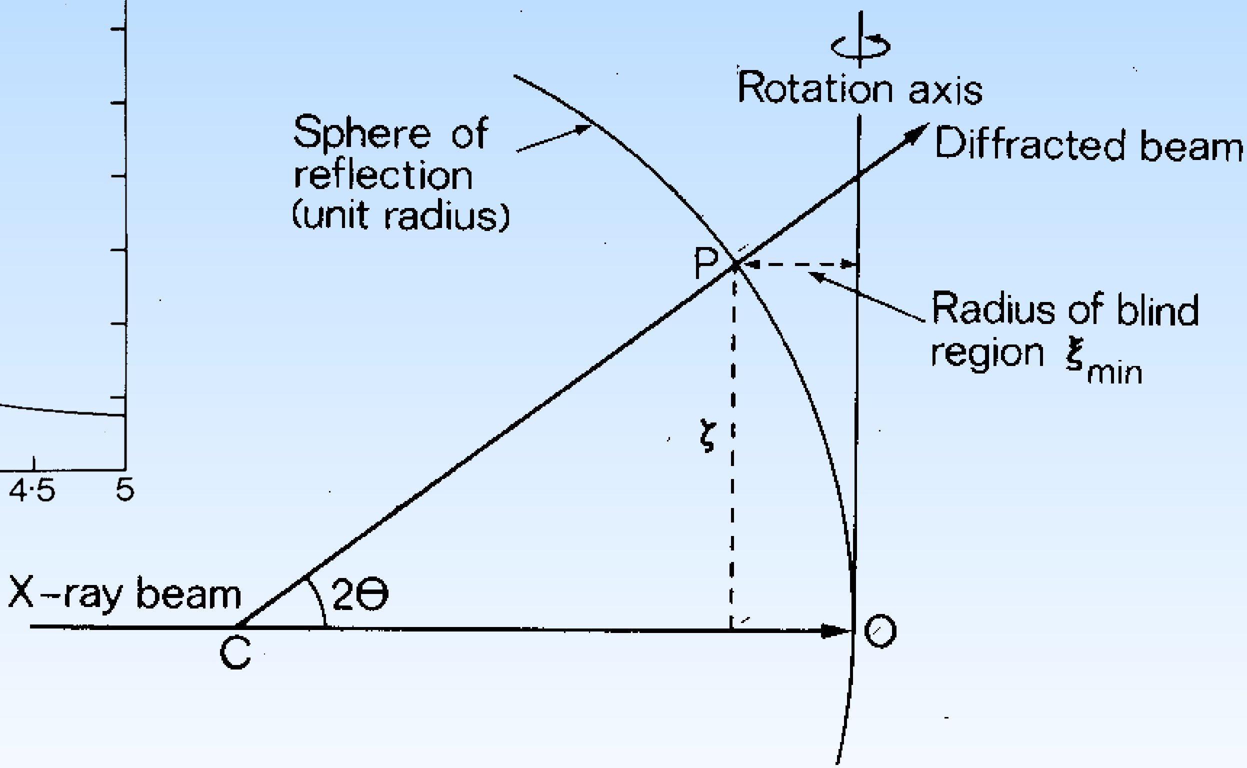
This leaves some reflections being clearly cut off.



Some data will be lost in the cusp of the torus



For λ = 1.5 Å



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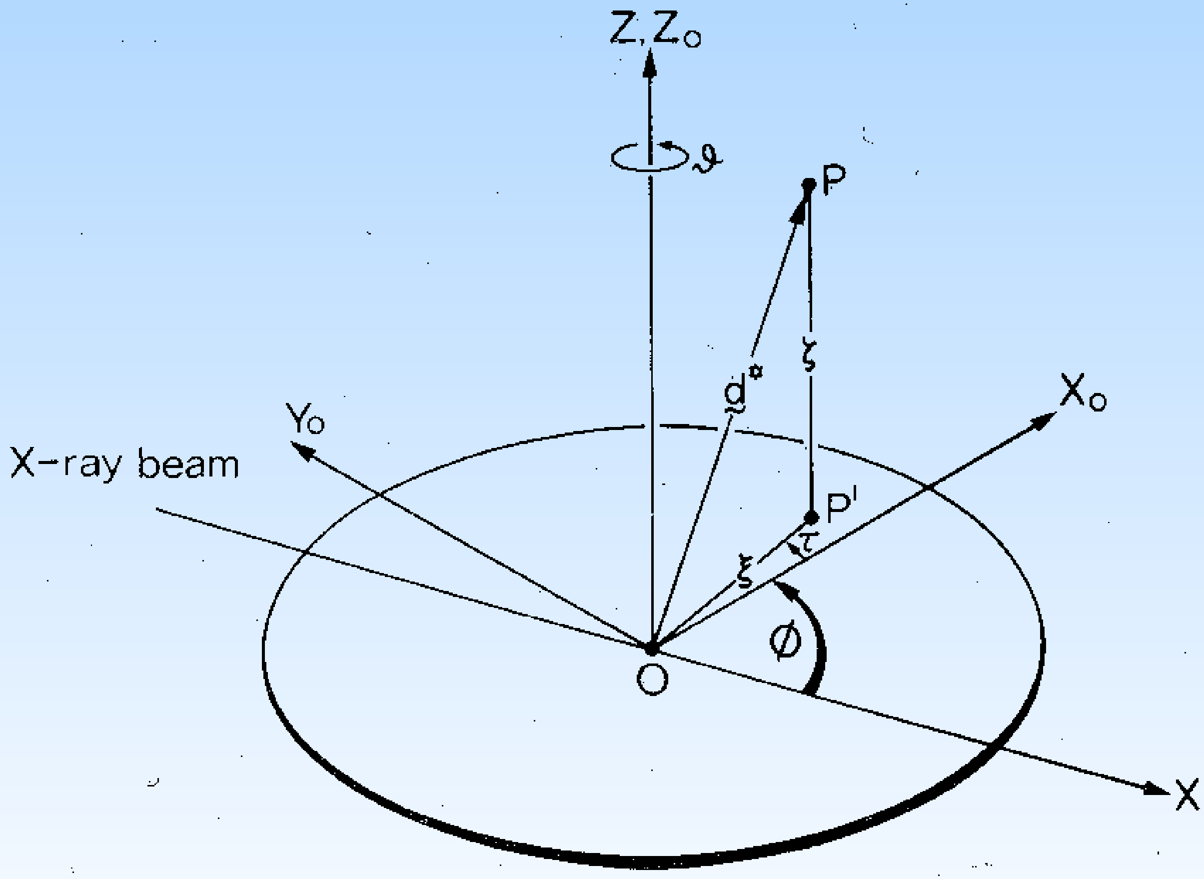
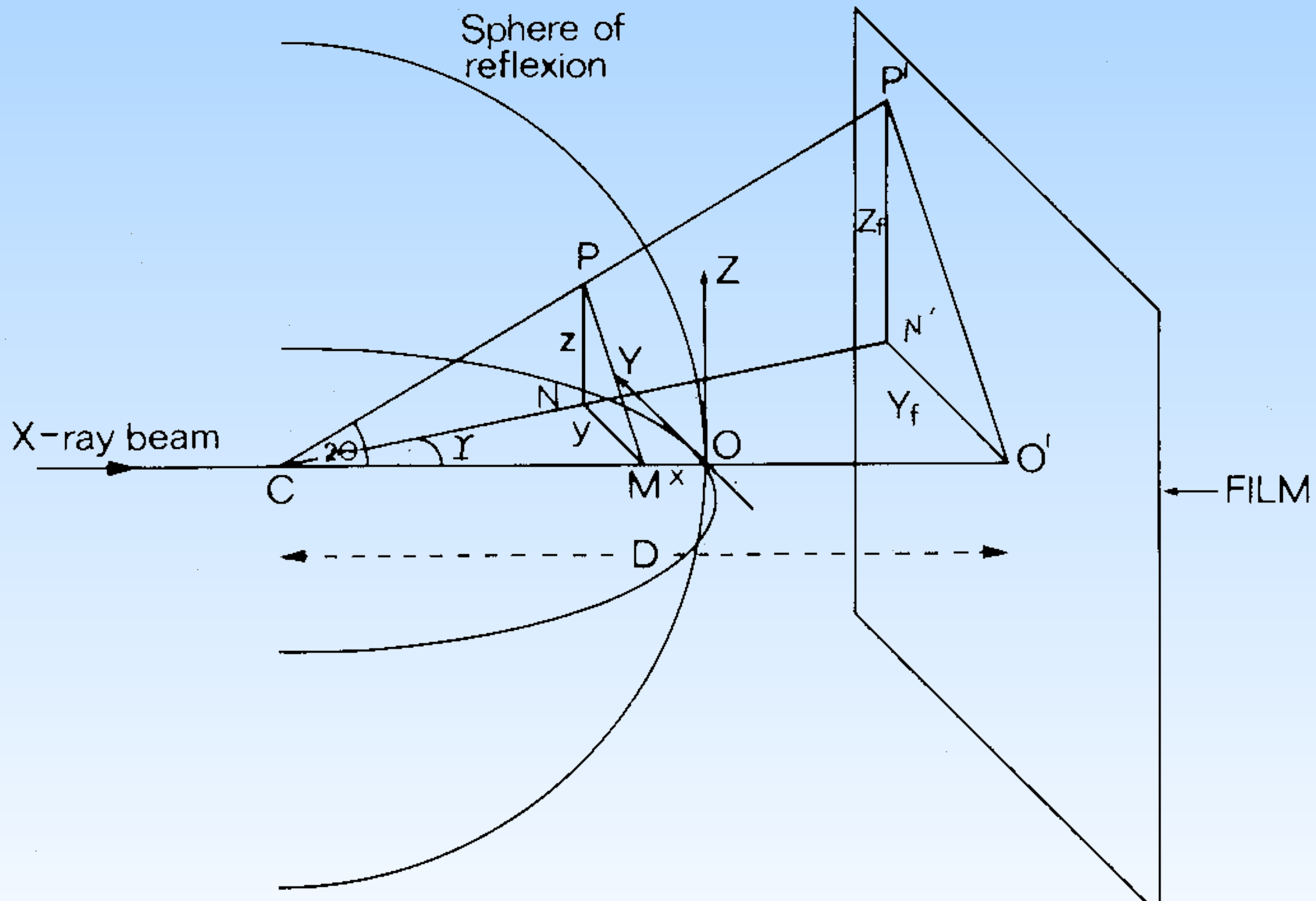
We can define the coordinates of a r.l. point in a spherical-polar system



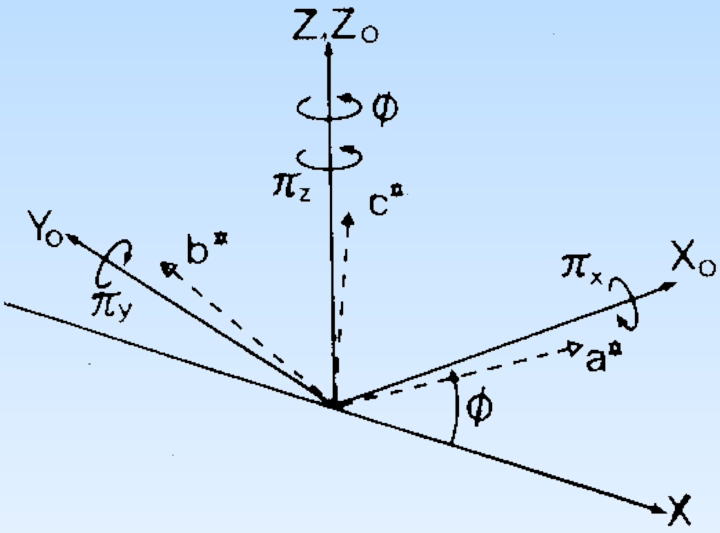
This can be nicely related to the coordinates of reflections on the film/detector



Finally, we need a way to describe the orientation of the crystal in the unit cell

- One scheme is to apply rotations π_x π_y π_z in order, then to apply axis rotation ϕ
- Sometimes ϕ is applied first
- Sometimes rot'ns are applied to *the new* position after rotation.
- In general, one ends up with matrix operations:

$$\mathbf{x}' = \mathbf{\Phi\Pi x}$$

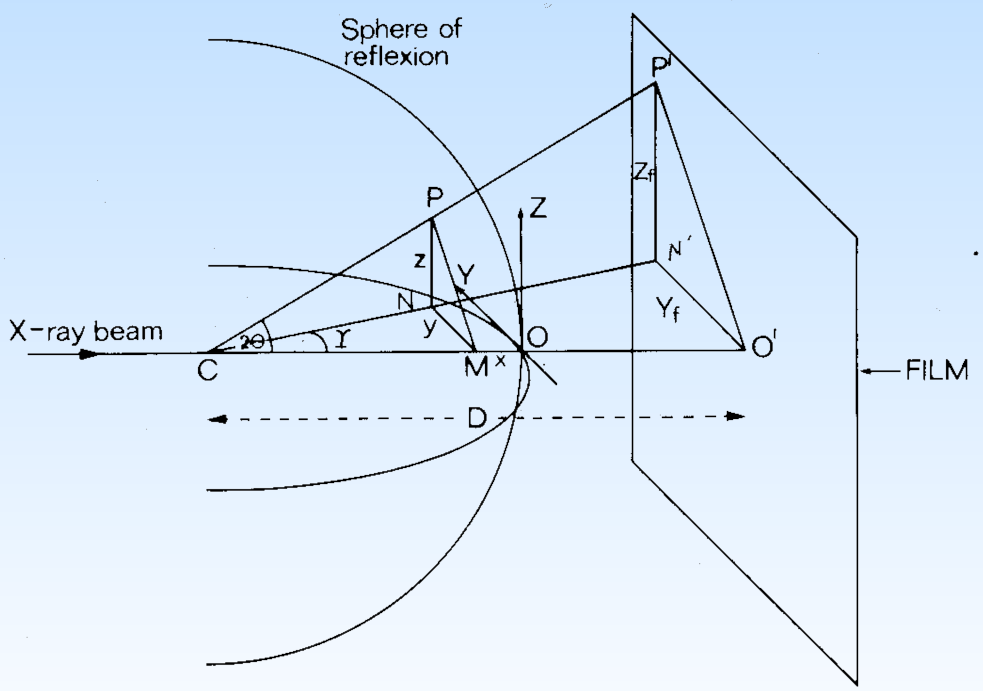


How do we process our data?

- If we know how the crystal is oriented, we can predict where to find reflections on the area detector
- We can measure intensities around each reflection
  - Integrate a box
  - Estimate Background
  - Evaluate integrated intensity
- How do we know the orientation?

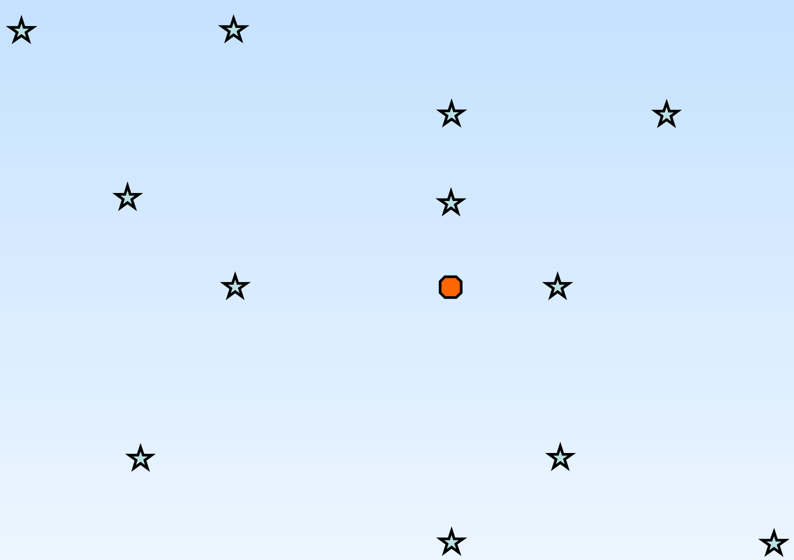
Autoindexing:

- For each reflection on the detector, we can project back to a point in the reciprocal lattice.
- Use some trick to guess the indices.
- Derive unit-cell parameters and crystal setting from that.

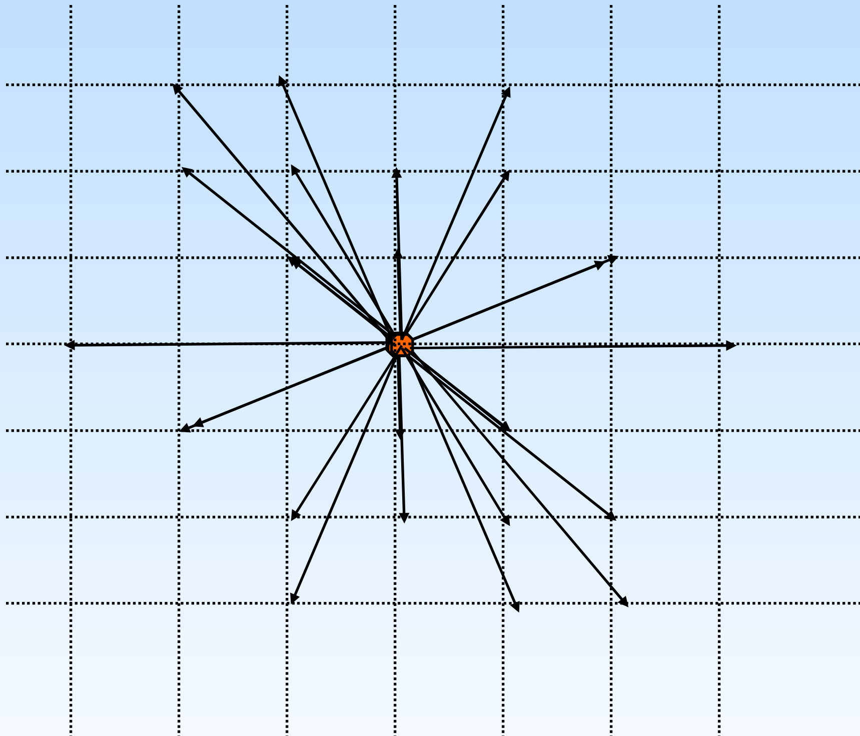


Vector-based Indexing - I

One finds a constellation of observed reflections

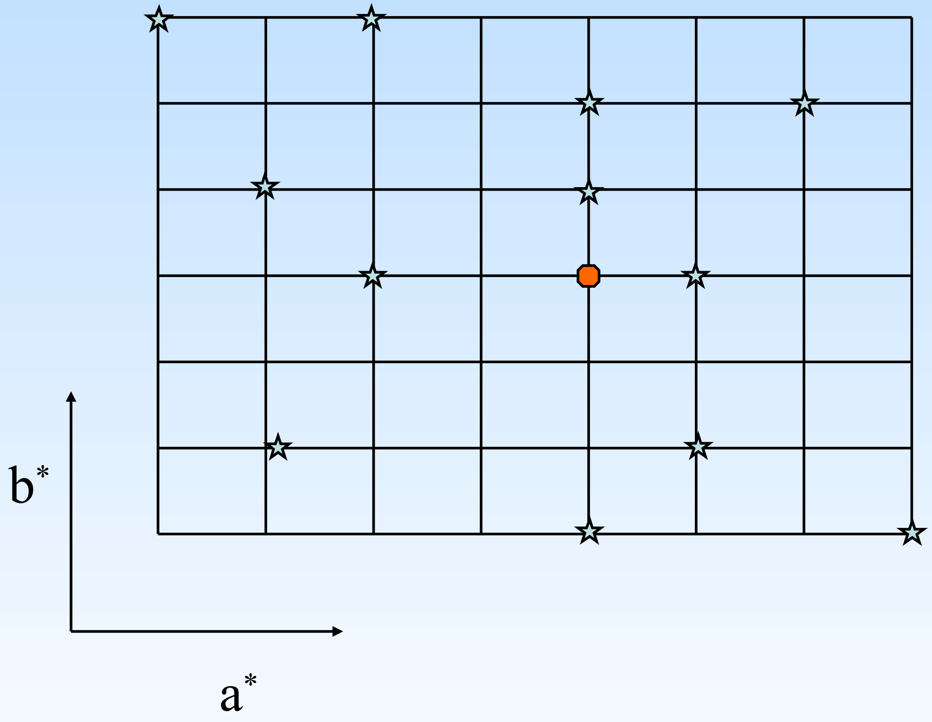


The vectors among them suggest a lattice



Vector-based Indexing - II

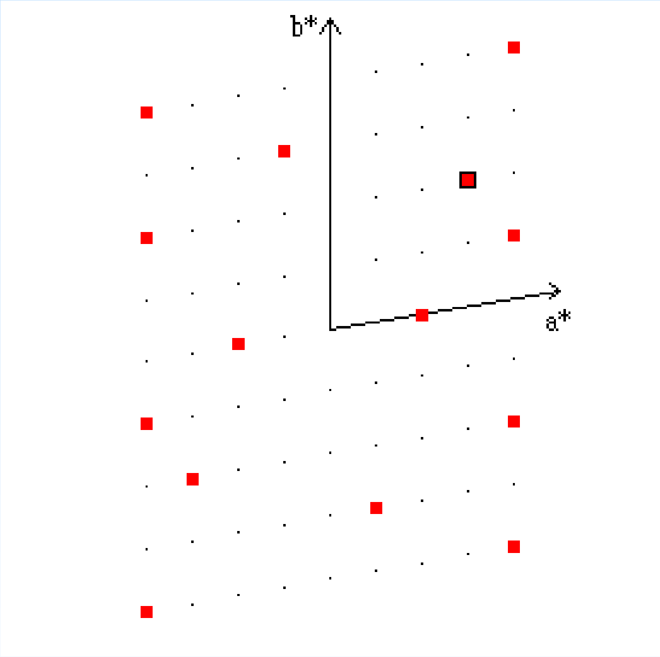
One can deduce the unit cell dimensions and orientation from the lattice

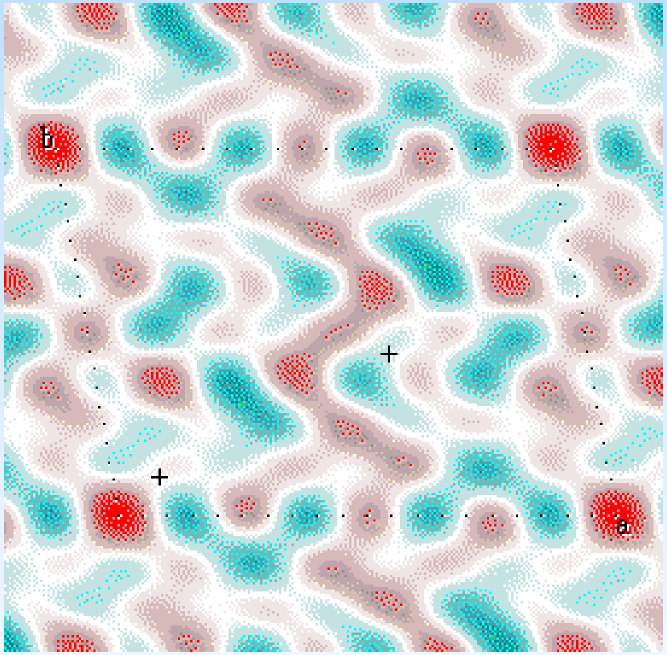


Fourier-based Indexing

- Again, one starts with a group of reflections
- Calculate a cosine Fourier transform of their positions

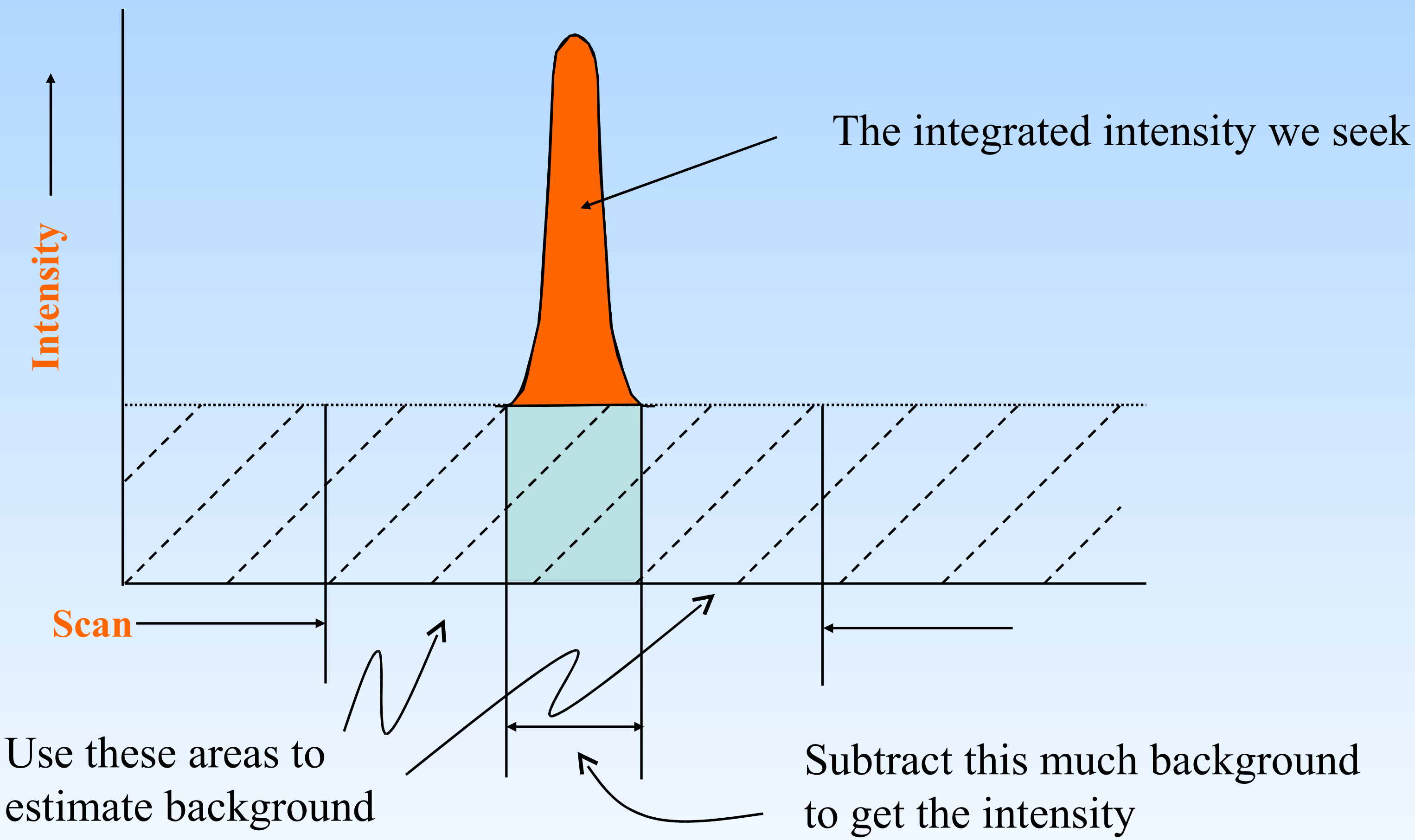
•The resulting “density” map gives a clear indication of the crystal lattice orientation and dimension



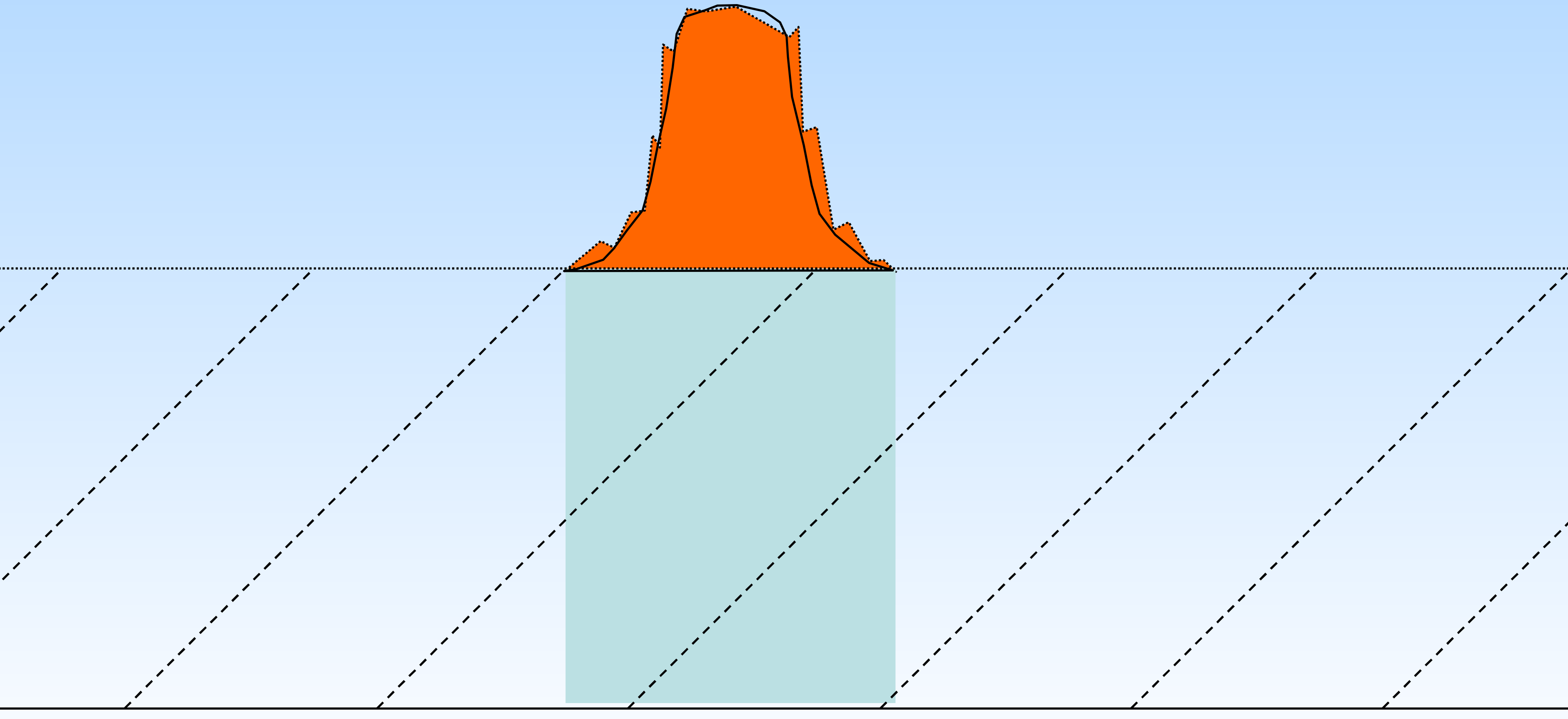


K. Cowtan -- Structure-Factor Applet

Integration of peaks



One can show that for weak reflections, it pays to fit *weak* reflections with a profile that is learned from the *strong* reflections.



The result for profile fitting is:

We normalize the learned profile so that $\sum p_i = 1$

Then if the Scan is S_i, the Background at each point is B_i, and the variance of each measurement is V_i, (usually S_i), then we have that the best estimate of intensity is:

$$I = \frac{\sum \frac{p_i(S_i - B_i)}{V_i}}{\sum \frac{p_i^2}{V_i}}$$

Summary:

- Folks have been struggling for years to understand the experiment well enough to analyze data in predictive mode.

- These days, the software knows much of this and we depend on that.